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Characteristics of a Low Fluoride Fish Protein Concentrate From Whole Croaker (Micropogon Undulatus).

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CHARACTERISTICS OF A LOW FLUORIDE FISH PROTEIN CONCENTRATE FROM WHOLE CROAKER (*MICROPOGON UNDULATUS*)

A Dissertation

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ABSTRACT

Fish protein concentrate made from whole fish has been approved as a food additive by the FDA and among its various standards a maximum concentration of 100 ppm of fluoride is permitted. FPC made from whole fish usually exceeds this limit and the use of deboned and eviscerated fish has become a necessary practice for the production of a low fluoride FPC.

The purpose of this study was to evaluate alternatives that would allow the use of whole fish without the need for deboning and eviscerating, which in addition to increasing costs and reducing production yields, are not always suitable practices for small fish such as industrial or trash fish.

Croaker, Micropogon undulatus, a representative of the Gulf of Mexico trash fish, a highly under-utilized resource, was selected as the raw material for this study. Several modifications to existing methods for FPC production were designed and evaluated for chemical composition by protein, fat, ash, moisture, phosphorus, calcium, magnesium, potassium, sodium, copper, manganese, zinc, iron and fluoride analyses; yields by total solids mass balances and solubility of the final products in water adjusted to different pH levels. Under the assumption that bones and scales are not soluble in isopropyl alcohol, a screening device was adapted for refining the defatted final product of three extractions

by separating the fine particles from the coarse fraction retained in the screen. This refining step proved to be effective in separating a 82% protein, 10% ash and less than 50 ppm fluoride fine fraction from a 70% protein, 20% ash and more than 100 ppm fluoride coarse fraction. It is suggested that if adapted to each extraction step, this screening modification would increase the yield of the fine fraction.

Classification of the dry solids of the coarse fraction by particle size (dry screening) proved to be less effective than separating the different bulk density fractions by air classification, which removed 10% of the total solids and up to 15% of the total ash.

The effect of solvent pH on the chemical composition and extraction efficiency was tested and it was found that adjusting the solvent pH to 4 or 10 was unnecessary since other chemicals were more soluble at these pH levels than fluoride, thereby increasing its relative concentration in the final products.

From the results and observations of this study, an alternative method for FPC production from whole fish is proposed. Employment of this method would retain some of the valuable minerals and in addition would also yield a high quality fish meal as a byproduct.

INTRODUCTION

Food production as a major concern has paralleled the demographic explosion. The gap between food supply and demand, and the shortage of high quality protein are increasing alarmingly. In order to minimize this deficiency it is necessary to maximize the utilization of all available food resources (Bressani, 1968; Rasmussen, 1969; Ehrlich and Ehrlich, 1970).

Fish is an excellent source of animal protein, but world fisheries are probably one of the most poorly managed natural resources, sometimes over-exploited, other times under-utilized, and often completely wasted. Predictions imply that fish landings could be doubled in the next 20 years without overfishing, but even now underfishing is not the only problem (Snyder, 1967). Under-utilization, wasting a portion of the catch and insufficient markets are other problems that aggravate the optimization of this resource (Russell, 1968). For example, it is estimated that in the United States alone, 660,000 metric tons of fish are under-utilized or discarded yearly (Bullis and Carpenter, 1968).

One method of preserving fish of limited economic value is its conversion into fish meal, a form of dehydrated fish in which original components are concentrated by wet reduction or dry rendering (Brody, 1965). Because of its high lipid content, fish meal characteristically has a fishy

odor and taste, brownish-grey color, and is difficult to store, which makes it acceptable only for animal and poultry feeds.

In contrast with fish meal, fish protein concentrate (FPC) was conceived as colorless and tasteless with good keeping properties. Ideally FPC is an inexpensive, organoleptically inert, highly nutritive protein supplement suitable for human consumption (Pariser, 1967; Nunn, 1969).

Trash or industrial fish, which has little or no economic value in the fresh market, and is used only on a relatively small scale for fertilizer, pet foods, and fish meal, has been suggested as the most economical raw material for human grade FPC production. However, these fish present many problems because the catch which is heterogeneous in size and species, is not always suitable for eviscerating and deboning, the common methods used to produce a low fluoride FPC (Morck, 1970).

Processing methods using whole fish require modification in order to reduce ash content, thus insuring an acceptable concentration of fluoride. The modified process must also be flexible enough to adapt to small batch or large scale continuous production, dependent upon the type and quantity of the fish supply. This may be furnished as a byproduct of shrimping and other fishing operations or landed specifically for industrial purposes.

The objective of this study was to evaluate modifications of existing production methods that could lead to a

more feasible and economic process for fish protein concentrate made from whole croaker Micropogon undulatus, one of the most abundant fish in the Gulf of Mexico.

Protein from the Sea

There has been a doubling in world population from 1.5 to 3 billion since the beginning of the century, and another doubling is anticipated by the year 2000 (Rasmussen, 1969).

In 1967 the President's Science Advisory Committee Panel on the World Food Supply estimated that 20 percent of the people in underdeveloped countries, which include approximately two-thirds of the world population, were not receiving enough calories per day and that 60 percent were seriously lacking in one or more essential nutrients, especially protein. This means 1.5 to as many as 2 billion people are either undernourished or malnourished. Of these, an estimated half billion can be described as either chronically hungry or starving. These numbers do not include the hungry and malnourished millions in the lower economic strata of developed countries such as the United States (Ehrlich and Ehrlich, 1970).

The recognition of wide-spread protein-calorie malnutrition in many developing nations led to the appointment in 1955 of a Protein Advisory Group (PAG) by WHO. In 1960 this group was expanded to the present tripartite FAO/WHO/UNICEF Protein Advisory Group. PAG provides a focal point for all aspects of the three agencies of the United Nations dealing with production, processing, safety, nutritive value, pediatric use, quality, and marketing of protein foods

(Kertesz, 1969).

Among 14 action proposals, PAG has recommended the development of an acceptable fish protein concentrate. Fishery resources, it is generally acknowledged, should have an especially important place in plans for closing the protein gap, both because of the high rank of fish protein in the nutritional value scale of foods, as well as the production cost advantage some fishery products have over other forms of protein of similar quality (Hamlish and Kreuzer, 1968).

Borgstrom (1970) described the last decade as a period of revolutionary change in world fisheries as a consequence of the new fleets of the major fishing nations equipped with modern electronic devices, new gear, and factory ships with sophisticated processing equipment.

In 1950, the total world catch of fish and shellfish was 20.2 million tons, a tenfold increment from 1850. By 1970, the total world production was 57 million tons (Chapman, 1966; FAO, 1970).

The maximum sustainable annual yield of food from the ocean has been estimated at 100 million tons (Ritmer, 1969), between 150 and 160 million tons (Ricker, 1965), more than 200 million tons (Holt, 1969) and 2 billion tons (Chapman, 1967).

It is not possible to predict the exact potential productivity of the sea, but fish protein concentrate is one logical alternative for improving the efficiency of today's resource (Christy, 1967). Fish reduction into fish meal has

increased from 1.5 to 15 million tons per year between 1948 and 1965, which represents approximately 50% of the world increment in fish landings, 20 to 55 million tons anually during the same period. Assuming a protein conversion factor of ten, some of these 3.7 million tons of animal feed could be utilized ten times more efficiently if they would be used directly for human consumption (Gulland and Carroz, 1968).

Fish Protein Concentrate as an Alternative

The term "fish protein concentrate" (FPC) was adopted by FAO in 1961 in preference to the earlier name "fish flour" to avoid confusion with cereal flours (FAO, 1961).

By definition fish protein concentrate is an inexpensive, stable, wholesome product of high nutritive quality, hygienically prepared from fish, in which the protein and other nutrient materials are more concentrated than in the fresh fish. This definition includes FPC products of varying characteristics ranging from tasteless, odorless, light-colored, flour-like materials, through coarse meals having a fishy taste and odor, to highly-flavored, dark-colored pastes or powders resembling meat extracts (Snyder, 1967).

This definition is quite arbitrary; it is restrictive and certainly not entirely satisfactory. It excludes for instance conventional fish meal, sun-dried and salted fish as well as a number of other very important products which for reasons of sanitation or nutritional value have not

been included. On the other hand, the definition includes certain highly flavored products such as fish sauces and pastes (Pariser, 1968).

"Whole fish" protein concentrate is prescribed as an additive by the Food Additive Regulation 121.1202 for use as a food supplement. It must meet the following specifications:

1. protein content- (N x 6.25) not less than 75% by weight of the final product;
2. protein quality- not less than 100;
3. moisture content- shall not exceed 10% by weight of the final product;
4. fat content- shall not exceed 0.5% by weight of the final product;
5. isopropyl alcohol residues- shall not exceed 250 ppm;
6. ethylene dichloride residues- shall not exceed 5 ppm;
7. fluorides (expressed as F)- shall not exceed 100 ppm;
8. microbial organisms- shall be free of Escherichia coli, and pathogenic organisms including Salmonella; and
9. total bacterial plate count- not more than 10,000 per gram of final product.

Production of concentrates from fish has been practiced for many years, reviews of earlier methods used before

the concept of fish protein concentrate have been reported (Pariser, 1967; Nunn, 1968).

FPC Production Methods

Processes used to manufacture fish and marine protein concentrates have been subdivided into three classifications; physical, biological and chemical (Knobl, 1967; Bertullo, 1968; Pariser, 1968).

There is not enough information about physical methods to properly evaluate them. They vary from processes such as the MIT-UNICEF method that yields a product for further solvent extraction using the Heat Transfer Medium (HTM) to dehydrate fish at low temperature under vacuum in about 1 1/2 hours, to more sophisticated techniques using electric discharges and electro-osmosis to separate fat from protein (Pariser, 1963).

Biological methods are based on fermentation principles and use microorganisms or enzymes natural to the fish or isolated from other sources to digest the protein into water soluble peptides and amino acids which are separated by filtration from the undigested material. After removing fat and oils by centrifugation the liquid portion is concentrated to about 50% solids and spray-dried, resulting in a crystalline powder (Levin 1950; Keyes and Meinke, 1962; Jeffreys and Krell, 1962; Bertullo, 1964; Hale, 1969).

Chemical methods use organic solvents to remove lipids and water from the raw material and at present are the only

practical chance of producing a low lipid FPC (Connell, 1969). Among the many solvents which have been used or proposed; hexane-ethanol (Allen, 1963), hexane vapors (Comtesse, 1968), ethyl alcohol (Galliver and Holmes, 1957; Dresoti et al, 1962) and acetone-water (Dambergs, 1956); only isopropyl alcohol and 1,2 dichloroethane have been approved by the FDA for FPC production (FDA, 1967).

The Viobin Corporation has developed a method for "fish flour" and fish meal production that utilized 1,2 dichloroethane azeotropic extraction (Swendsen, 1967; Ershoff and Rucker, 1969; Levin, 1969). This process has been extensively reviewed (Brody, 1965) and reached commercial production (Nunn, 1968). The Viobin process utilized a final extraction with isopropyl alcohol in order to remove 1,2 dichloroethane which produces a toxic choline derivate, chlorocholine chloride with an LD_{50} of approximately 500 mg/kg (Munro and Morrison, 1964).

Isopropyl alcohol is identified with the Canadian Halifax Process (Guttmann and Vandenheuvel, 1957; Power, 1963; Dambergs, 1969a; Idler, 1968) and in the United States with the Bureau of Commercial Fisheries method to produce marine protein concentrate (BCF, 1966; Brown and Miller, 1969).

The water-isopropyl alcohol azeotrope has been recommended as a practical and economical solvent system for fat extraction (Dambergs, 1969b). Guttmann et al.(1967) and Dubrow and Hammerle (1969) have shown that fish can be preserved in isopropyl alcohol prior to processing.

FPC and Fluoride

The term fluoride is used to denote the ionic form and the inorganic form in which fluorine has combined with other elements. Fluorine is the seventeenth in order of abundance among the elements and constitutes approximately 0.03 percent of the earth's crust. It is found principally in the forms of fluorospar (CaF_2), cryolite (Na_3AlF_6), and fluoroapatite ($Ca_5F(PO_4)_3$) (Larget, 1961; Jolly, 1966).

The classification of fluorine as an essential or a nonessential element depends upon the criteria employed in determining essentiality. If an essential element is considered one that must be provided in the diet to permit survival, then fluorine cannot yet be regarded as essential for plants, microorganisms, or animals. If an essential element is defined as one which is ordinarily required for health and well-being under the usual conditions in which individuals live, then fluorine must be considered as an essential element in human nutrition (Underwood, 1971).

Sharpless and McCollum (1933), in an attempt to demonstrate the indispensability of fluorine in the diet, fed three generations of rats a very low fluoride diet and found that growth and reproduction were not affected, and that fluoride depletion was almost accomplished without apparent tooth damage or alterations. However, the incidence of rats losing their tails during the three first weeks was higher than in the control group and a slight proliferation of capillaries in the tooth pulp and surrounding bone was

noted in the low fluoride groups. However, since these conditions had been noted at times in stock animals, they were not attributed to the lack of fluorine alone.

Shaw (1967) has reviewed the rapid progress made in knowledge about the effects of human consumption of flourine on many aspects of health and reported the following:

> Controlled water fluoridation, artificial or natural, at an optimal corrected level of 0.7 to 1.1 ppm is a highly effective public health procedure for reduction of dental caries, a costly and prevalant disease.
>
> Fluoridation could also reduce the incidence of osteoporosis (indicated by decreased bone density and collapsed vertebrae). In addition, it may lessen calcification of the abdominal aorta.
>
> Of 904 necropsies performed in Colorado Springs where the water contains 2.5 ppm fluoride, 334 upon subjects who had lived there for more than 20 years, evaluation of the pathologic findings revealed no evidence that prolonged exposure to water containing fluoride at this level had been harmful to any organ system.

A review of fluorolysis in man and acute and chronic fluoride toxicity in man and animals was made by Largent (1961). Normal blood fluoride levels range up to 0.050 mg/100 ml. In fatal cases, blood fluoride levels may be as high as 0.2 to 0.3 mg/100 ml. Excretion of fluoride in

the urine is about 1.0 mg/24 hr. Even subtoxic doses of fluoride can result in sharp increases in urine levels, and in severe poisoning, the urine concentration can be several milligrams/ 100 ml (Tietz, 1970).

In New Zealand, a comparative study showed more caries in a community with high fluoride in the drinking water than in a second community with low fluoride. Analysis of the soils and edible parts of the vegetables grown in the second community showed differences in composition from similar samples taken from the high fluoride area. The largest differences involved the element molybdenum. Not only could differences in food be detected, but deciduous teeth from the low caries community had elevated molybdenum levels; however, no differences could be shown in permanent teeth or hair. Similar suggestive findings have come from Hungary where water levels of molybdenum have been correlated with low caries incidence in both high and low fluoride areas (Myers, 1971).

Very few foods contain more than 1-2 ppm fluoride and most of them less than 0.5 ppm (dry basis). Fluoride concentration of 100 ppm in tea are common, 2/3 of which passes into the infusion. One cup of tea increases the fluorine of the diet by 0.1 to 0.2 mg. As much as 1 mg fluorine can be ingested daily by adults in some communities from this source alone (Underwood, 1970).

Values for the availability of fluoride from FPC have been reported at 93% as available as NaF in adult male

humans (NaF absorption was 94% and fluoride from FPC, 88% (Spencer et al., 1970), and 25 to 52% as available as NaF in weanling female rats (Zipkin et al., 1970). Spencer attributed the difference in values to species differences and/or to an age effect. Stillings et al. (1971) corrected the calcium, phosphorus, and fluoride levels of these previous diets and postulated that the difference in values was partially due to differences in the amounts of these elements between the two diets.

The desirability of keeping fluoride levels in FPC below 100 ppm, as prescribed by the FDA, is based on the possibility of tooth mottling rather than on definite pathological hazard. This has encouraged most investigators to incorporate methods for removing bones from their product, since the skeletal system is the main contributor of fluoride. This practice has thus reduced the total mineral content of FPC (Liston and Pigott, 1970). Fluoride concentration in FPC made from whole fish varies with species and season (Table 1).

Eviscerated and/or deboned fish rather than whole fish has become the most common raw product fro FPC production. The Swedish company Nabisco-Astra named its product eviscerated fish protein (EFP) (Lawler, 1970). Since EFP is 90% protein it better accomplishes the ideal of FPC as a protein supplement, and even though the fluoride content is well below 100 ppm it is at the expense of other minerals which are also nutrients and should be considered as part

Table 1

Fluoride Concentration in FPC
Made from Whole Fish

Species	Fluoride Concentration (ppm)	Location
Hake	210	a
Red Hake (Containing 13% ash)	150 to 300	b
Cod	143 to 240	c
Herring	123 to 189	c
Capelin	57	c
Dog Fish	761	c
Skate	372	c
Anchovy	171	d
Pacific hake	426	d
Pacific herring	140	d
Menhaden	88	d

a). BCF College Park, Maryland (Spencer et al., 1970).
b). BCF College Park, Maryland (Zipkin et al., 1970).
c). Halifax Laboratory, Nova Scotia, Canada (Ke et al., 1970).
d). National Marine Fisheries Service Technology Laboratory NOAA; Seattle, Wash. (Spinelli et al., 1971).

of the supplement. Deboning and eviscerating fish, in addition to increasing production costs create a waste disposal problem, and trash fish do not lend themselves to automatic deboning because of their small size.

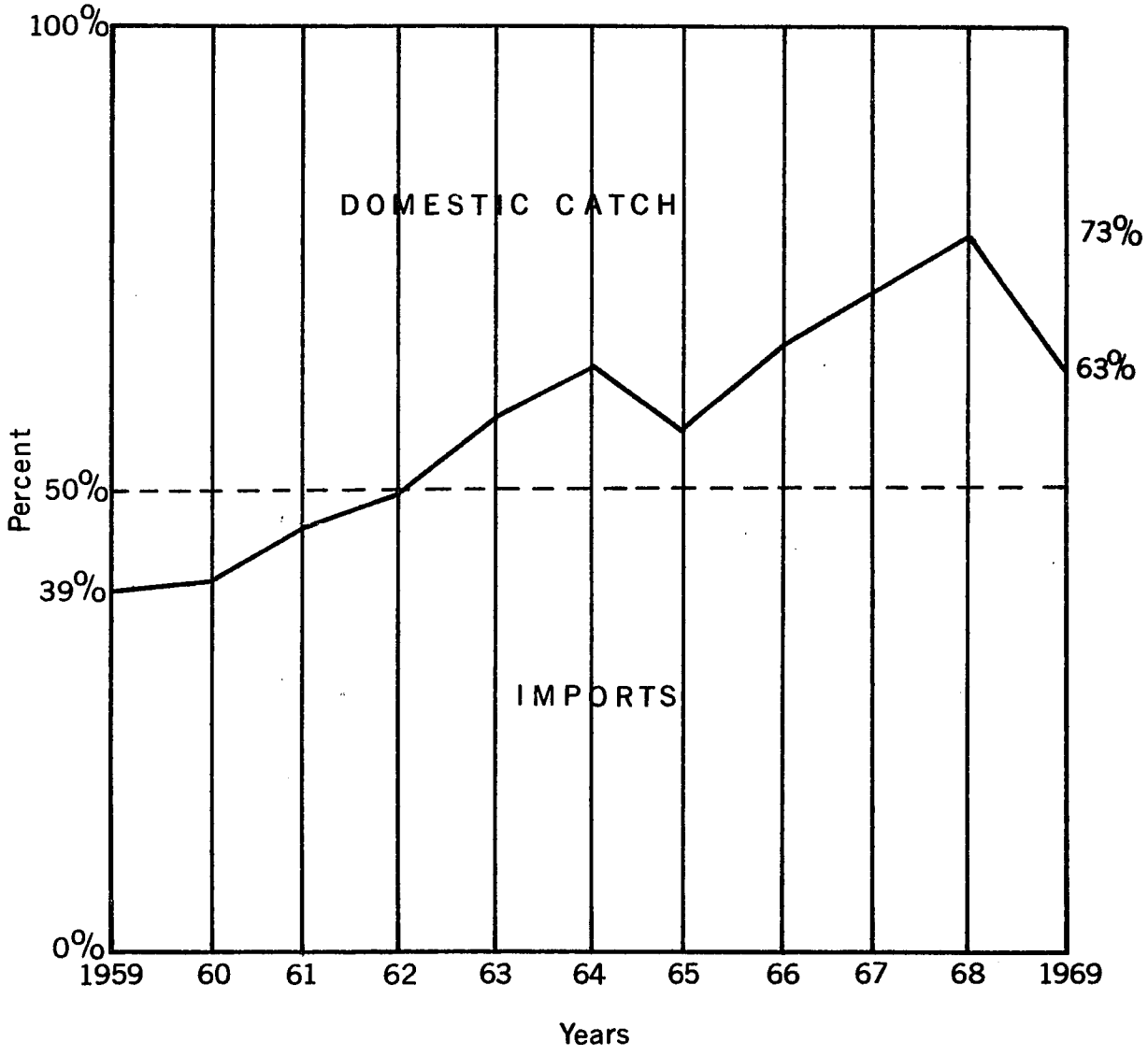
Hyder and Cobb (1972) stated that during 1967 the catch of "trash fish" or "industrial bottom fish" in the Gulf of Mexico was 48,762 metric tons, and another 660,317

metric tons were discarded during shrimping operations.

The waste of fish in the Gulf of Mexico represents approximately 10% of the total domestic and imported fisheries supply of the United States. After ranking first among fishing nations in the world, the United States has now become an importer of fish (Figure 1).

Figure 1

Percent Domestic Catch and Percent Imports of the U.S. Supply and Consumption of Fishery Products (1959-1969)*



* U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service. Fishery Statistics of the United States, 1969, Washington, D.C.

MATERIALS AND METHODS

Introduction

Croaker (Micropogon undulatus), which has a proximate composition varying from 4.5 to 10% fat, 16.5 to 17.1% protein, 2.37 to 6.42% ash and 67.1 to 79.5% moisture (Thompson, 1959a, 1959b, 1959c), represents up to 50% of the Gulf of Mexico trash or industrial fish by weight (Compton, 1969) and has proven to be a good raw material for FPC production (Loustaunau, 1971, Hyder, 1972). This fish was processed into fish protein concentrate by modifying the Bureau of Commercial Fisheries method for particle size of the raw product, solvent concentration, extraction temperature and the inclusion of refining steps.

Under the assumption that bones and scales are not soluble in isopropyl alcohol, the fish was comminuted into larger particles (3/8 inch instead of 1/8 inch). A wet screening device was adapted to separate the fine solids, mainly protein, from the coarse fraction containing much of the bones and scales during an additional step. Further refining was accomplished by sieving and air classification of the coarse fraction (Hoskins and Loustaunau, 1973).

Azeotropic isopropyl alcohol (87.8% instead of 91%) was selected as the solvent concentration and the extraction temperature was lowered from 78°C to 65°C in order to facilitate and simplify solvent handling, anticipating continuous commercial operation.

Quality and efficiency of the process were evaluated by proximate and mineral analyses. Fluoride determination served as the indicator of fractionation effectiveness. Mass balance, solvent recuperation and solubility data were also collected.

Raw Material

A 250 pound lot of fresh croaker (Micropogon undulatus, Linnaeus) was randomly taken from the unloading conveyer of a commercial trawler that supplied trash fish to the Tabby Cat Food Company of Golden Meadow, Louisiana. The fish was packed in polyethylene bags and transported in ice to the Baton Rouge laboratory, where after being classified (Breder, 1948; Eddy, 1969) it was stored in the freezer.

The complete batch of whole frozen Croaker was comminuted into particles 3/8 inch or smaller by use of a Hobart 1/2 HP commercial meat grinder. The frozen, ground fish was divided into homogeneous samples of approximately 1500 grams. The samples, packed in double polyethylene bags, were randomly assigned to each treatment and kept frozen at -20°C until processed.

Solvent

The binary azeotrope AIPA (87.8% isopropyl alcohol-12.2% water) used for all extractions was prepared from bulk anhydrous isopropyl alcohol distilled with water, or recuperated from previous extractions. The solvent recuperation method (Fig. 6) consisted of the following: the

liquid phase of the filtration was vacuum distilled and passed through an ion exchange resin (Amberlite IR-120 A.R.) in order to remove volatile organic bases and other impurities; the solvent was then redistilled and the azeotropic isopropyl alcohol (boiling point 80.4°C) was collected. Solvent pH was adjusted with NaOH or HCl when necessary.

Experimental Methods

EXTRACTION METHOD: In the extraction unit (Fig. 2), 1250 grams of ground whole croaker were combined with 3125 ml of azeotropic isopropyl alcohol. A one hour extraction was carried out under continuous mechanical agitation at 65°C. Following extraction the liquid phase, mainly solvent with lipids and water, was separated from the fish solids by vacuum filtration.

Two additional consecutive extractions of fat and water were made in the same manner by recombining the fish solids with 3125 ml of fresh AIPA (Fig. 4).

REFINING: The "wet screening" unit (Fig. 3) was made from a 5000 ml pyrex bottle cut 8 inches high with an inside cylindrical basket (6 inches in height and 5 inches in diameter) made of #20 wirescreen, where the solids of the final extraction mixed with approximately 2000 ml of AIPA were stirred for 5 minutes. This separation was repeated 4 times, or until no more fine solids passed through the screen.

Figure 2

Extraction Unit Used for FPC Production

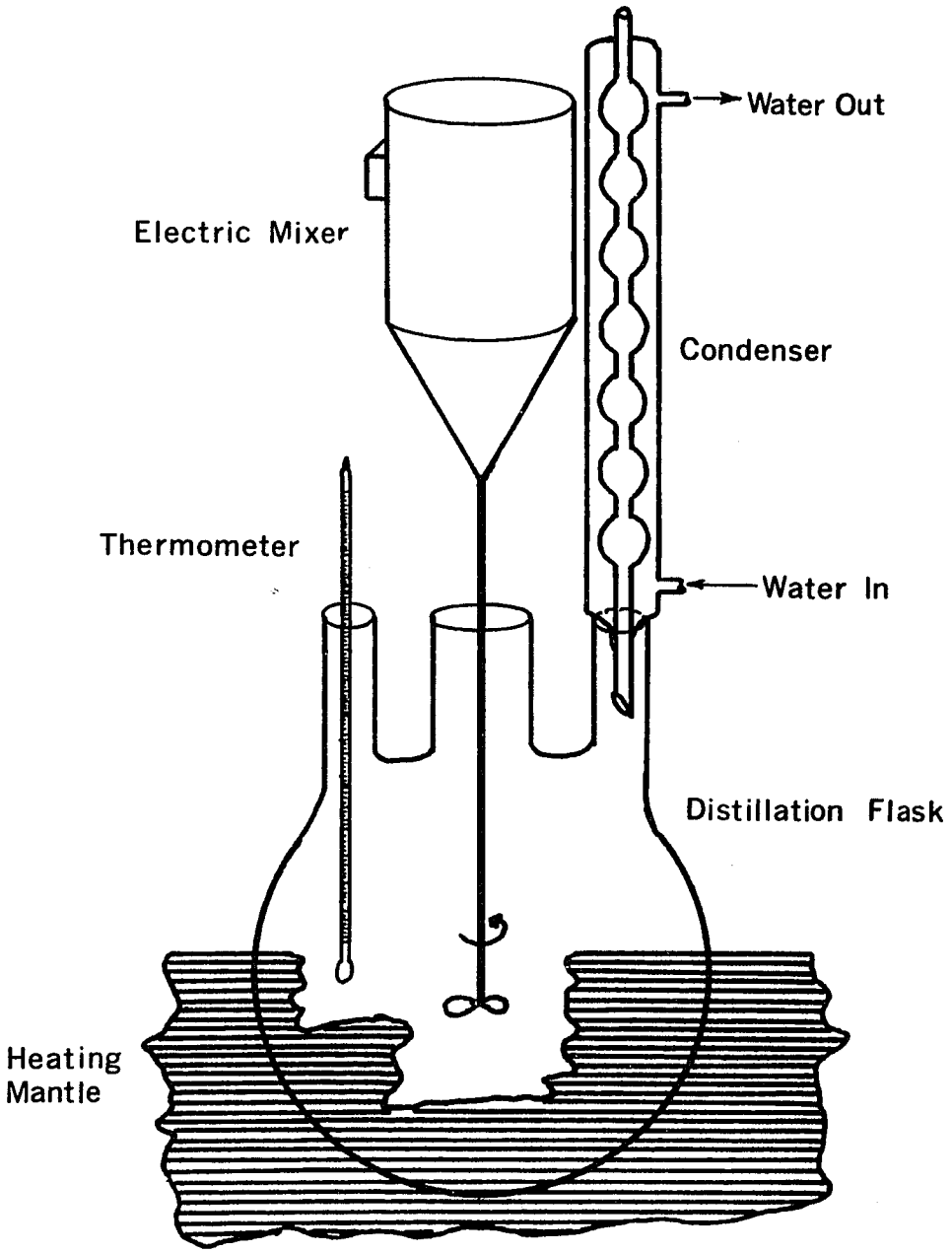


Figure 3

Wet Screening Unit Used to Separate Fine Particles from the Coarse Material

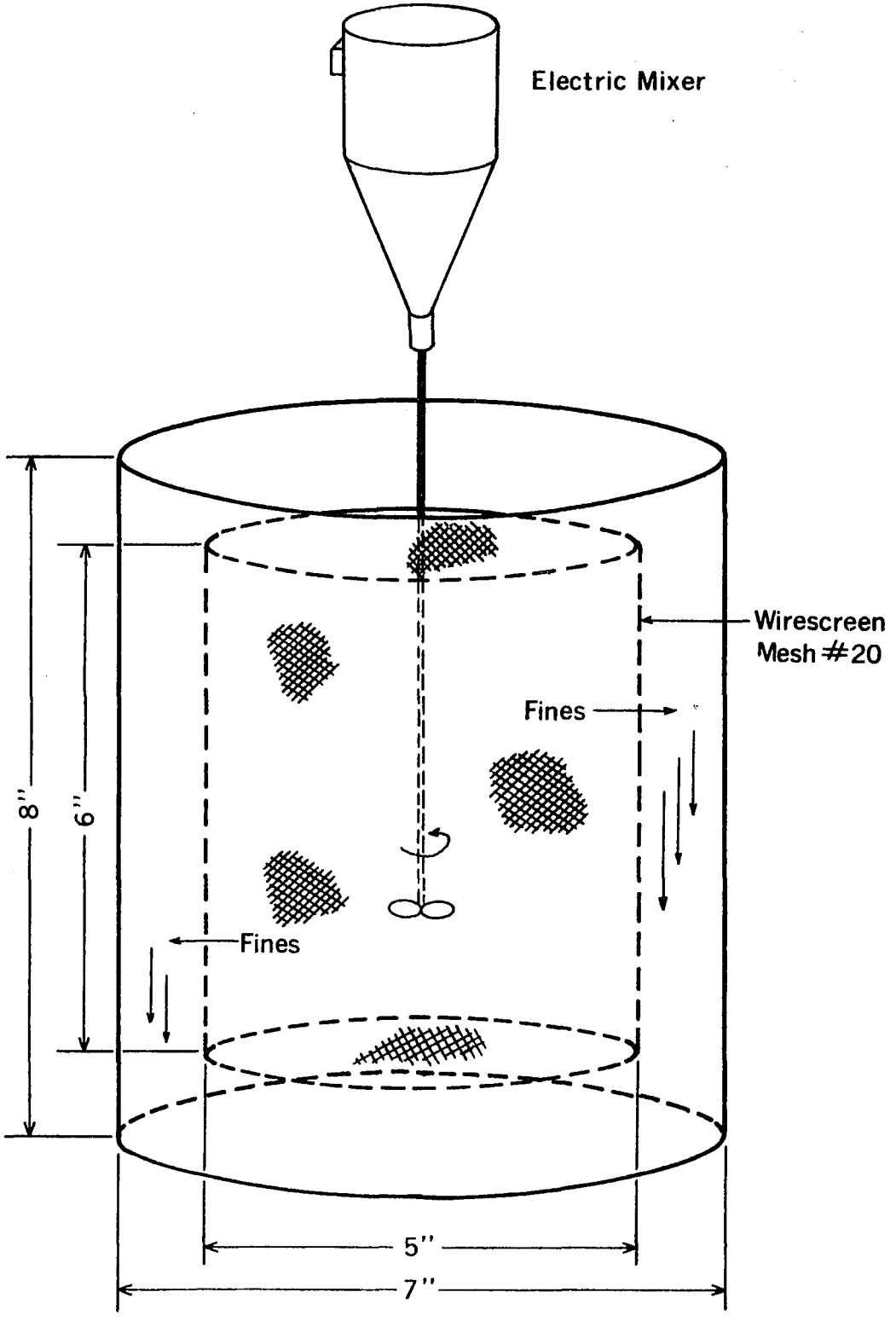
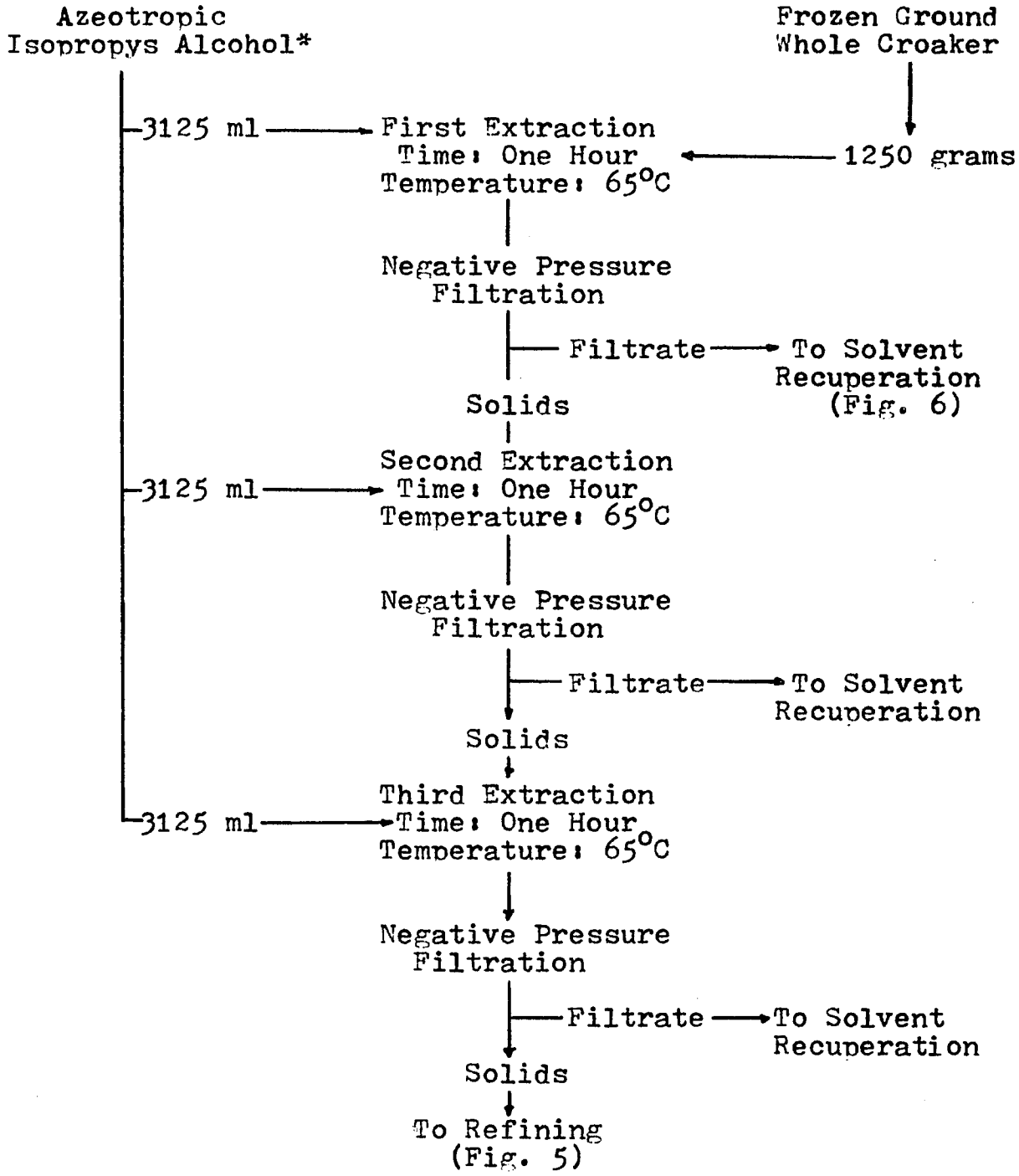


Figure 4

Flow Diagram for the Preparation of FPC
Extraction Method



* Solvent pH was adjusted with NaOH or HCl.

Figure 5

Flow Diagram for Refining of FPC
by Wet Screening and Air Classification

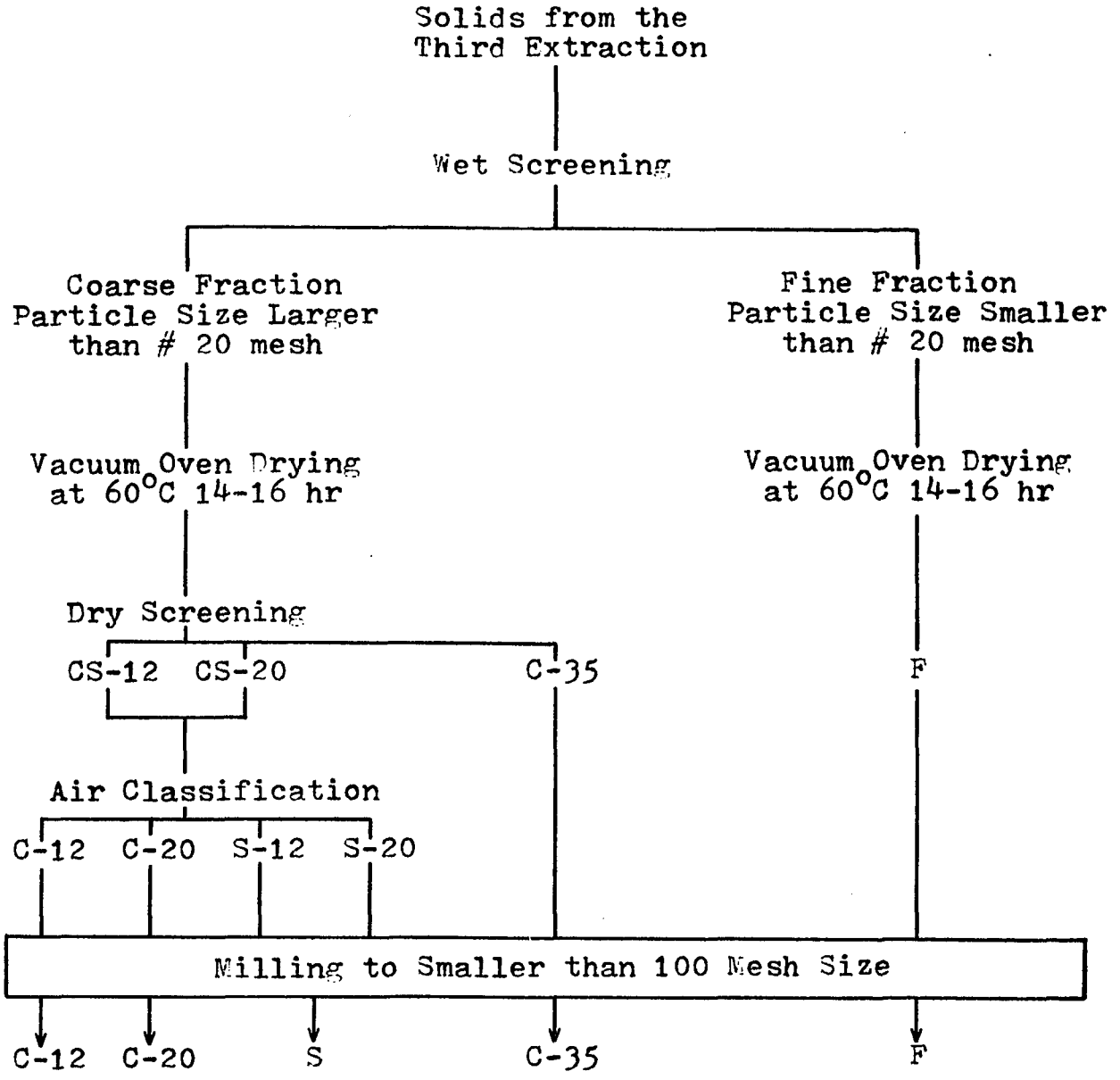
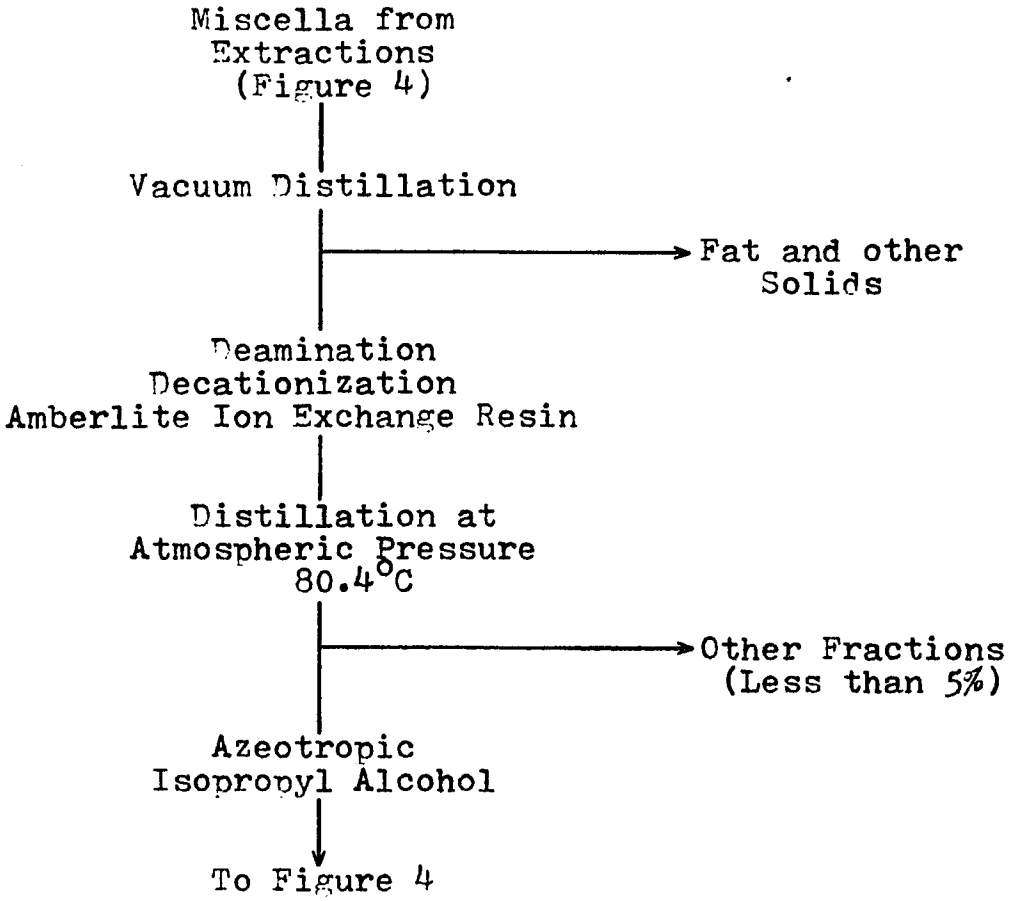


Figure 6

Solvent Recuperation for Recycling
Flow Diagram



DRY SCREENING: In separate trays, both the fine and coarse products of the wet screening process were vacuum dried for 14 to 16 hours at 60°C. The dry coarse fraction was placed in a portable sieve shaker (Tyler Model Rx 8) with three U.S. Standard Sieves of 12, 20 and 35 mesh sizes. After shaking for 15 minutes, the coarse fraction was separated into three subproducts referred to as CS-12, CS-20 and CS-35. The fine product was referred to as F (Fig. 5).

AIR CLASSIFICATION: The coarse products CS-12 and CS -20 were further refined by using an air classifier (E. L. Erickson, Model B). The low density fraction, mostly scales with some powder, S-12 and S-20, was separated from the higher density portion C-12 and C-20.

MILLING: For analytical purposed all samples were milled and homogenized to # 80 mesh powder using a micro-mill (Laboratory, Wiley Intermidiate 1/2 HP).

Experimental Design

TREATMENTS: In an attempt to determine the effectiveness of the refining modifications to the processing method (Figs. 4 and 5) and to study the effect of the solvent (AIPA) pH, the following four treatments were designed:

1. a control treatment utilized dry screening at pH 7 (DS-7) which did not include the wet screening and air classification steps (Fig. 5) was compared to the modified process;

2. a second treatment consisted of wet screening at

pH 7 (WS-7) and was extracted with solvent at pH 7 followed by wet screening and air classification;

3. the third treatment was wet screening at pH 4 (WS-4) extracted with solvent (adjusted to pH 4 with HCl), and refined in the same manner as the previous treatment; and

4. the fourth treatment involved wet screening at pH 10 (WS-10) extracted with AIPA (adjusted to pH 10 with NaOH) and similarly refined (Table 2).

REPETITIONS: Three independent runs of each treatment were made with samples randomly assigned to each treatment-repetition. Analyses of each product were made in duplicate.

Analytical Methods

The raw material, products of first and second extractions, and all five fractions were analyzed for proximate and mineral composition.

Fat, moisture, ash and protein (N x 6.25) analyses were made according to methods of the Association of Official Analytical Chemists (AOAC, 1970).

For minerals, one gram of sample was wet-ashed with three parts of HNO_3 to one part $HClO_4$ and diluted to volume. Calcium, magnesium, potassium, sodium, copper, manganese, zinc and iron were determined on a Jarrell-Ash Atomic Absorption Spectrophotometer, and phosphorus was determined by the colorimetric, vanadomolybdate method

Table 2

Nomenclature and Coding of Treatments Used and Products Obtained

Products	Treatments			
	Dry Screening pH-7	Wet Screening pH-4	Wet Screening pH-7	Wet Screening pH-10
	DS-7	WS-4	WS-7	WS-10
Solids of:				
raw product	RP	RP	RP	RP
first extraction	1x	1x	1x	1x
second extraction	2x	2x	2x	2x
Final coarse fractions retained by:				
sieve # 12	CS-12	C-12	C-12	C-12
sieve # 20	CS-20	C-20	C-20	C-20
sieve # 35	CS-35	C-35	C-35	C-35
Final fine product of wet screening	F	F	F	F
Low density fraction of air classification		S	S	S

with the aid of a Technicon Auto-Analyzer (AOAC, 1970).

Fluoride content was determined in the raw and final products by the specific ion electrode method, using an Orion Model 404 specific ion meter equipped with an Orion Model 94-09 fluoride solid-membrane electrode and a standard calomel reference electrode (Ke et al., 1970).

Solubility

For solubility tests of the products of the four treatments, C-12, C-20 and C-35 were pooled into one coarse fraction and compared with the fine fraction F. The determinations were made by placing one gram of sample into a 200 ml beaker and adding 100 ml of water which had been previously adjusted to the correct pH with NaOH or HCl. One hour extractions of soluble nitrogen were made with a magnetic mixer followed by filtration through Whatman No. 1 filter paper and collection of the filtrate.

Fifty ml of the filtrate were pipetted into a standard Kjeldahl flask for nitrogen determination (AOAC, 1970). Solubility results were expressed as mg of nitrogen from 1 gram of sample soluble in 100 ml of water.

RESULTS AND DISCUSSION

The effect of solvent pH and refining modifications on the chemical composition, total solids mass balance and solubility properties of the different products obtained by the treatments (extraction methods) tested are presented as follows.

Analytical results of the determinations of the 14 chemical composition parameters (protein, fat, ash, moisture, phosphorus, calcium, magnesium, potassium, sodium, copper, manganese, zinc, iron and fluoride) of the products are recorded in Tables 21 through 40 in the Appendix, and summarized as the average of six determinations (duplicate analyses of three runs) in Tables 3 through 10. The total solids mass balance for each individual run are presented in Figures 7 through 18 and values for nitrogen solubility at different pH levels are shown in Tables 19 and 20.

Chemical composition

Differences in chemical composition among all the products were statistically analyzed using 4 sets of analyses of variance (ANOV) with randomized complete-block designs (Steel, 1960). Orthogonal comparisons (Snedecor, 1969) were used in order to break down the variation into its sources.

The first two sets of 13 ANOV were designed to detect the overall effect if any of solvent pH and refining modi-

Table 3

Average* Percent Composition of Dry Screening pH-7 Products

Products	% Solids	% Moisture	% Protein	% Fat	% Ash
Raw Product- wet basis	30.05	69.95	16.47	9.82	3.82
Solids of raw product	96.27	3.73	52.78	31.67	12.37
Solids of first extraction	95.63	4.37	58.12	24.43	13.25
Solids of second extraction	94.53	5.47	73.58	3.73	17.20
Final coarse fraction retained by sieve # 12	91.74	8.26	70.66	0.32	21.80
Final coarse fraction retained by sieve # 20	91.24	8.76	69.85	0.32	21.88
Final coarse fraction retained by sieve # 35	92.33	7.67	76.00	0.20	16.66
Final fine fraction	92.35	7.65	83.00	0.44	8.93

* Average of three runs and duplicate analyses, Tables 21 and 22 in Appendix.

Table 4

Average* Percent Composition of Wet Screening pH-4 Products

Products	% Solids	% Moisture	% Protein	% Fat	% Ash
Raw Product- wet basis	32.19	67.81	17.46	10.94	3.96
Solids of raw product	97.90	2.10	53.10	33.28	12.04
Solids of first extraction	97.65	2.35	57.40	28.28	12.90
Solids of second extraction	95.67	4.33	66.73	13.76	14.96
Final coarse fraction retained by sieve # 12	94.40	5.60	73.38	2.09	21.21
Final coarse fraction retained by sieve # 20	94.22	5.78	75.23	1.41	18.69
Final coarse fraction retained by sieve # 35	94.68	5.32	80.07	1.15	12.42
Final fine fraction of wet screening	96.86	3.14	84.06	1.09	9.92
Low density fraction of air classification	93.76	6.23	67.41	0.47	26.82

* Average of three runs and duplicate analyses, Tables 23 and 24 in Appendix.

Table 5

Average* Percent Composition of Wet Screening pH-7 Products

Products	% Solids	% Moisture	% Protein	% Fat	% Ash
Raw Product- wet basis	31.29	68.71	16.93	10.71	3.80
Solids of raw product	97.81	2.19	54.05	32.03	12.27
Solids of first extraction	96.16	3.84	63.25	20.62	14.41
Solids of second extraction	96.55	3.45	76.95	2.90	16.65
Final coarse fraction retained by sieve # 12	93.41	6.59	74.50	0.46	19.91
Final coarse fraction retained by sieve # 20	94.30	5.70	74.83	0.22	19.75
Final coarse fraction retained by sieve # 35	94.33	5.67	79.00	0.19	14.73
Final fine product of wet screening	94.84	5.16	84.67	0.12	10.22
Final coarse fraction of air classification	93.72	6.28	65.63	0.16	28.47

* Average of three runs and duplicate analyses, Tables 25 and 26 in Appendix.

Table 6

Average* Percent Composition of Wet Screening pH-10 Products

Products	% Solids	% Moisture	% Protein	% Fat	% Ash
Raw Product- wet basis	30.47	69.54	17.21	9.71	3.89
Solids of raw product	95.44	4.56	57.44	29.69	12.42
Solids of first extraction	95.27	4.73	57.33	25.60	19.94
Solids of second extraction	95.03	4.97	63.20	16.41	14.80
Final coarse fraction retained by sieve # 12	92.88	7.12	71.81	1.18	18.89
Final coarse fraction retained by sieve # 20	93.59	6.41	73.88	0.55	19.33
Final coarse fraction retained by sieve # 35	92.67	7.33	78.16	0.45	13.50
Final fine fraction of wet screening	93.63	6.38	82.35	0.29	10.18
Low density fraction of air classification	92.13	7.87	67.01	0.55	26.35

* Average of three runs and duplicate analyses, Tables 27 and 28 in Appendix.

Table 7

Average* Mineral Composition of Dry Screening pH-7 Products

Product	P %	Ca %	Mg %	K %	Na %
Raw Product- wet basis	0.68	0.99	0.04	0.27	0.14
Solids of raw product	2.17	3.18	0.13	0.88	0.45
Solids of first extraction	2.44	3.93	0.15	0.63	0.36
Solids of second extraction	3.36	5.27	0.19	0.74	0.46
Final coarse fraction retained by sieve # 12	4.10	6.50	0.19	0.75	0.52
Final coarse fraction retained by sieve # 20	4.25	6.33	0.20	0.78	0.55
Final coarse fraction retained by sieve # 35	3.10	4.36	0.18	0.70	0.46
Final fine fraction	1.37	2.01	0.18	0.67	0.36

Table 7

(continuation)

Product	Cu ppm	Mn ppm	Zn ppm	Fe ppm	F ppm
Raw Product- wet basis	2.4	5.7	10.8	55.4	26.4
Solids of raw product	7.8	18.5	34.7	176.7	84.3
Solids of first extraction	14.5	22.5	75.2	191.7	-
Solids of second extraction	12.2	25.2	87.8	203.3	-
Final coarse fraction retained by sieve # 12	20.2	25.2	86.7	146.7	222
Final coarse fraction retained by sieve # 20	22.0	26.2	84.8	148.3	189
Final coarse fraction retained by sieve # 35	23.2	20.8	94.7	178	135
Final fine fraction	44.7	15.3	119.2	457	39

* Average of three runs and duplicate analyses, Tables 29, 30 and 31 in Appendix.

Table 8

Average* Mineral Composition of Wet Screening pH-4 Products

Products	P %	Ca %	Mg %	K %	Na %
Raw Product- wet basis	0.69	1.05	0.04	0.28	0.11
Solids of raw product	2.19	3.25	0.12	0.86	0.35
Solids of firse extraction	2.40	3.54	0.13	0.46	0.22
Solids of second extraction	2.92	4.34	0.16	0.44	0.23
Final coarse fraction retained by sieve # 12	3.77	5.93	0.19	0.41	0.24
Final coarse fraction retained by sieve # 20	3.54	5.41	0.19	0.40	0.24
Final coarse fraction retained by sieve # 35	2.50	3.54	0.17	0.37	0.20
Final fine fraction of wet screening	1.86	2.93	0.19	0.38	0.18
Low density fraction of air classification	5.48	7.21	0.24	0.56	0.37

Table 8

(continuation)

Products	Cu ppm	Mn ppm	Zn ppm	Fe ppm	F ppm
Raw Product- wet basis	2.1	4.9	13	41	26
Solids of raw product	6.5	15.2	41.2	128.3	81.2
Solids of first extraction	7.0	17.0	40.3	150.0	-
Solids of second extraction	8.8	19.7	47.2	175.0	-
Final coarse fraction retained by sieve # 12	14.8	26.8	55.6	155.0	338.2
Final coarse fraction retained by sieve # 20	15.5	25.0	63,7	158.0	259.2
Final coarse fraction retained by sieve # 35	15.3	18.0	70.0	185.0	128.5
Final fine fraction of wet screening	34.5	18.5	74.7	436.7	48.0
Low density fraction of air classification	26,7	30.7	59.2	168.0	180.5

*Average of three runs and duplicate analyses, Tables 32, 33 and 34 in Appendix.

Table 9

Average* Mineral Composition of Wet Screening pH-7 Products

Products	P %	Ca %	Mg %	K %	% Na
Raw Product- wet basis	0.71	1.06	0.03	0.29	0.12
Solids of raw product	2.25	3,38	0.12	0.92	0.36
Solids of first extraction	2.51	3.96	0.15	0.69	0.30
Solids of second extraction	3.26	4.57	0.18	0.77	0.36
Final coarse fraction retained by sieve # 12	3.23	5.02	0.17	0.70	0.34
Final coarse fraction retained by sieve # 20	3.35	4.92	0.18	0.64	0.34
Final coarse fraction retained by sieve # 35	3.09	4.36	0.17	0.67	0.32
Final fine fraction of wet screening	1.78	2.82	0.19	0.68	0.30
Low density fraction of air classification	5.73	7.37	0.21	0.80	0.44

Table 9

(continuation)

Products	Cu ppm	Mn ppm	Zn ppm	Fe ppm	F ppm
Raw Product- wet basis	2.3	4.65	11	53	26
Solids of raw product	7.5	14.8	34.8	168.3	87.3
Solids of first extraction	9.5	18.0	46.5	190	-
Solids of second extraction	9.8	22.8	61.7	231.6	-
Final coarse fraction retained by sieve # 12	20.7	21.7	76.7	201.7	222.2
Final coarse fraction retained by sieve # 20	18.3	26.2	66.3	168.3	261.2
Final coarse fraction retained by sieve # 35	16.0	20.5	67.8	190.0	159.2
Final fine fraction of wet screening	45.7	19.7	100.0	478.5	45.7
Low density fraction of air classification	28.0	28.3	73.3	150.8	205.7

* Average of three runs and duplicate analyses, Tables 35, 36 and 37 in Appendix.

Table 10

Average* Mineral Composition of Wet Screening pH-10 Products

Products	P %	Ca %	Mg %	K %	Na %
Raw Product- wet basis	0.69	1.06	0.04	0.25	0.12
Solids of raw product	2.31	3.48	0.13	0.83	0.39
Solids of first extraction	2.70	4.21	0.15	0.45	0.25
Solids of second extraction	2.32	4.78	0.14	0.39	0.23
Final coarse fraction retained by sieve # 12	3.53	5.60	0.19	0.41	0.27
Final coarse fraction retained by sieve # 20	3.73	5.87	0.19	0.40	0.28
Final coarse fraction retained by sieve # 35	2.88	4.10	0.18	0.37	0.24
Final fine fraction of wet screening	2.10	2.58	0.20	0.36	0.21
Low density fraction of air classification	4.79	7.21	0.26	0.54	0.38

Table 10

(continuation)

Products	Cu ppm	Mn ppm	Zn ppm	Fe ppm	F ppm
Raw Product- wet basis	2.6	4.4	11	45	26
Solids of raw product	8.5	14.5	36.0	147	84.7
Solids of first extraction	5.3	18.0	38.8	173	-
Solids of second extraction	7.2	17.8	41.0	170	-
Final coarse fraction retained by sieve # 12	8.2	23.2	51.7	193	251.5
Final coarse fraction retained by sieve # 20	7.2	25.0	45.7	173	244
Final coarse fraction retained by sieve # 35	29.2	19.7	62.5	236	141.8
Final fine fraction of wet screening	21.0	18.8	67.5	385	44
Low density fraction of air classification	15.8	23.5	58.8	165	197

* Average of three runs and duplicate analyses, Tables 38, 39 and 40 in Appendix.

fications on each of these 13 chemical components of the products: protein, fat, ash, moisture, phosphorus, calcium, magnesium, potassium, sodium, copper, manganese, zinc and iron.

Set 1 of ANOV involved the four treatments (DS-7, WS-4, WS-7 and WS-10) and seven products (RP, 1x, 2x, C-12, C-20, C-35 and F) in a RCBD arrangement. This set was run to determine the overall effect of solvent pH and screening methods on the chemical composition of the products involved (Table 41).

F values for Set 1 of ANOV are given in Table 12 and show that the differences among the means of all the components analyzed were highly significant for treatments with the exception of phosphorus, whose concentration in the final products was not affected by pH. All 13 F values for the components of the products were also highly significant and the interaction, products x treatments, was highly significant in all the analyses except ash and fat, indicating that the differences in concentrations of components in the various products were not in the same order of magnitude from treatment to treatment.

In an attempt to detect whether the differences in the main components; protein, ash and fat were due to the solvent pH, the screening method or to both, three sets of orthogonal comparisons were made among treatments (Table 12). The first orthogonal comparison indicated that the differences between the protein, ash and fat means of the

Table 11

F Values for Set 1 of ANOV Involving Four Treatments and their Products, Excluding the Low Density Fraction

ANOV for:	Treatment	Product	Prod x Treat	Residual
Protein	12.2**	283 **	4.51**	22.8**
Fat	11.9**	446 **	4.60**	112.6**
Moisture	16.5**	16.2**	0.81	142.6**
Ash	5.1**	92.6**	1.47	34.3**
Phosphorus	2.7	71.1**	4.21**	30.3**
Calcium	17.6**	412 **	13.7 **	6.3**
Magnesium	14.8**	266 **	12.85**	4.9**
Potassium	1675 **	623 **	43.81**	21.1**
Sodium	1634 **	192 **	40.44**	5.8**
Copper	175.4**	750 **	54.43**	14.0**
Manganese	28.2**	181 **	14.76**	10.1**
Zinc	442 **	340 **	24.35**	11.2**
Iron	44.1**	1272 **	18.08**	21.1**
d.f.	3 and 56	6 and 56	18 and 56	56 and 84
$F_{.05}$	3.36	2.65	2.01	1.62
$F_{.01}$	4.16	3.14	2.28	1.77

** $P < .01$

Table 12

Sum of Squares of the Orthogonal Comparisons Involving the Four Extraction Methods and Their Products, Except the Low Density Fraction

Comparison	d.f.	S.S. Protein	S.S. Fat	S.S. Ash
Among treatments				
1. DS-7, WS-7 vs WS-4, WS-10	1	87.58**	302.60**	53.10**
2. WS-7 vs DS-7	1	222.46**	9.01	7.42
3. WS-4 vs WS-10	1	30.72**	20.42	0.00
Treatment sum of squares	3	340.76	332.03	60.52
Among products				
1. RP, 1x, 2x vs C-12, C-20 C-35 and F	1	10605.84**	18511.42**	213.82**
2. RP vs 1x, 2x	1	1900.23**	3457.53**	89.38**
3. 1x vs 2x	1	1475.19**	2894.64**	76.43**
4. C-12, C-20, C-35 vs F	1	1409.81**	0.93	1278.16**
5. C-12, C-20 vs C-35	1	447.32**	1.64	544.64**
6. C-12 vs C-20	1	8.84	1.78	3.53
Products sum of squares	6	15847.23	24868.14	2225.96

** $P < .01$

two methods at pH 7 (dry screening, pH-7 and wet screening, pH-7) and the two methods at different pH (wet screening, pH-4 and wet screening, pH-10) were highly significant. However, in a second comparison between wet screening and dry screening at pH 7, it was found that the mean for protein of WS-7 was significantly higher than that of DS-7, but no significant differences could be detected among the mean values for fat and ash. A third comparison involving the other two methods failed to detect any significant difference between the means for ash and fat of wet screening at pH 4 and pH 10.

Among the seven products, three groups of six orthogonal comparisons were employed for protein, ash and fat. Upon breaking down the Sum of Squares into its individual components, it was shown that approximately 88% of the variation was due to the differences among the three products RP, 1x and 2x and their difference from the final products, C-12, C-20, C-35 and F (Table 12).

The differences between the coarse fractions, C-12, C-20 and C-35, and the final fine products (F) showed the highly significant effect of wet screening on the reduction of ash content and the concentration of protein.

Set 2 of ANCV was designed to test the overall interaction between solvent pH and wet screening and to determine the effect of air classification; this set excluded DS-7 removing the variation due to dry screening alone. The three wet screening treatments and all their products

were arranged in a RCBD and 13 ANOV for the same variables as Set 1 were made (Table 41).

The F values for Set 2 of ANOV are presented in Table 13. All the differences of the means of each chemical were highly significant for treatments, products and interactions.

Orthogonal comparisons among treatments were designed to determine the effect of pH on the means of protein, ash and fat of the three wet screening extraction methods (Table 14). Comparison 1 between wet screening at pH 7 and wet screening at pH's 4 and 10 showed that the means for protein, ash and fat of the treatment carried out at pH 7 were highly significantly different from the acid and basic methods. The second comparison between treatments WS-4 and WS-10 failed to detect any significant differences between the means of the parameters tested.

Seven orthagonal comparisons were made for protein, ash and fat among the products (Table 14). Comparisons 1, 2 and 3 show that the highly significant differences among RP, 1x and 2x and between these products and the final products accounted for over 75% of the variation of protein, over 99.96% of the variation of fat and 68% of the sum of squares for ash.

Since more than half of the variation among the products was due to the raw product and the products of the first two extractions, two additional sets of 14 ANOV were designed removing this source to find the variation if any

Table 13

F Values for Set 2 of ANOV Involving Wet Screening Treatments and All their Products

ANOV for:	Treatment	Product	Prod x Treat	Residual
Protein	200.8**	3104 **	75.2**	21.7**
Fat	1738 **	40107 **	608 **	98.2**
Moisture	1659 **	1410 **	81.0**	142 **
Ash	124 **	4594 **	26.0**	4.7**
Phosphorus	36 **	342.7**	115 **	33 **
Calcium	36.1**	2157 **	35.1**	26.1**
Magnesium	14.3**	322.1**	14.2**	8.6**
Potassium	1916 **	22.9**	26.0**	26.5**
Sodium	1748 **	1017 **	58.0**	38.1**
Copper	161 **	467 **	65.0**	14.7**
Manganese	22.5**	243.8**	15.9**	10.7**
Zinc	130.9**	156.3**	12.4**	9.5**
Iron	56.8**	856.0**	18.7**	24.5**
d.f.	2 and 48	7 and 48	14 and 48	48 and 72
$F_{.05}$	3.99	2.57	2.14	1.69
$F_{.01}$	5.93	3.40	2.72	1.96

** $P < .01$

Table 14

Sum of Squares of the Orthogonal Comparisons Involving the Wet Screening Treatments and all their Products

Comparisons	d.f.	S.S. Protein	S.S. Fat	S.S. Ash
Among treatments				
1. WS-7 vs WS-4, WS-10	1	198.34**	229.66**	29.48**
2. WS-4 vs WS-10	1	29.48	17.53	0.09
Treatment sum of squares	2	227.82	247.19	29.57
Among Products				
1. RP, 1x, 2x vs C-12, C-20, C-35 and F	1	7308.11**	16062.79**	630.09**
2. RP vs 1x, 2x	1	1241.67**	2264.72**	58.09**
3. 1x vs 2x	1	835.21**	1710.17**	37.72**
4. C-12, C-20, C-35, F vs S	1	1734.49**	2.00	1896.17**
5. C-12, C-20, C-35 vs F	1	872.82**	1.70	760.69**
6. C-12, C-20 vs C-35	1	316.56**	4.29	439.67**
7. C-12 vs C-20	1	18.06	2.38	5.05
Products sum of squares	7	12326.94	20051.04	3827.47

** P .01

among the means of the individual components of the final products.

Sets 3 and 4 were composed of fourteen analyses of variance for the same 13 variables as Sets 1 and 2 with the inclusion of fluoride. Set 3 of ANOV involved the four treatments and the products C-12, C-20, C-35 and F (Table 41).

F values for Set 3 of ANOV are presented in Table 15. The differences among the means for protein, fat, ash, potassium, sodium, copper, zinc, iron and fluoride of the four treatments were highly significant. The differences among the chemical components of the products were also highly significant except for magnesium and potassium which remained relatively constant in all products and the interaction products x treatments was highly significant for phosphorus, calcium, sodium and copper.

Orthogonal comparisons for protein, ash and fat were designed in order to detect the magnitude of the effect of solvent pH and screening methods on the composition of the final products (Table 16).

A comparison of dry screening versus wet screening showed the protein to be significantly higher and the ash lower in wet screening products; fat was significantly higher in the wet screening products, however wet screening at pH 7 was significantly more efficient in fat extraction than than at pH 4 or 10. Fat, ash and protein compositions were not significantly different between WS-4 and WS-10.

Table 15

F Values for Set 3 of ANOV Involving Four Treatments and the Final Products C-12, C-20, C-35 and F

ANOV for:	Treatment	Product	Prod x treat	Residual
Protein	7.23**	77.94**	0.82	24.01**
Fat	28.66**	5.82**	1.20	30.00**
Ash	20.67**	3.73**	1.01	78.79**
Moisture	3.38	120.24**	1.74	38.23**
Phosphorus	3.34	116.66**	4.33**	25.11**
Calcium	2.09	100.0 **	3.30**	4.56**
Magnesium	2.90	0.46	1.35	4.56**
Potassium	56.22**	0.91	0.29	24.92**
Sodium	218.70**	28.50**	3.95**	14.00**
Copper	8.79**	33.34**	4.30**	15.87**
Manganese	0.05	14.60**	1.02	13.40**
Zinc	25.80**	15.10**	1.09	10.40**
Iron	150.00**	62.00**	0.91	26.20**
Fluoride	5.51**	138.00**	3.40**	12.20**
d.f.	3 and 32	3 and 32	9 and 32	32 and 48
$F_{.05}$	3.56	3.56	2.54	1.87
F.01	4.46	4.46	3.02	2.21

** $P < .01$

Table 16

Sum of Squares of the Orthogonal Comparisons Involving the Four Extraction Methods and the Final Products C-12, C-20, C-35 and F

Comparisons	d.f.	S.S. Protein	S.S. Fat	S.S. Ash
Among treatments				
1. DS-7 vs WS-4, WS-7, WS-10	1	128.53**	13.24**	45.00**
2. WS-7 vs WS-4, WS-10	1	12.37	8.11**	6.26
3. WS-4 vs WS-10	1	32.01	0.07	0.05
Treatment sum of squares	3	172.91	21.42	51.31
Among products				
1. C-12, C-20, C-35 vs F	1	1409.81**	0.93	1278.34**
2. C-12, C-20 vs C-35	1	447.32**	1.64	544.64**
3. C-12 vs C-20	1	8.84	1.78	3.54
Products sum of squares	3	1865.97	4.35	1826.52

** P .01

Testing the products revealed the final fine product F to be highest in protein and lowest in ash, and C-35 was significantly higher in protein and lower in ash than C-12 and C-20. C-12 and C-20 were not significantly different from each other except in fat content. Because of the large particle size of C-12, the efficiency of extraction was greatly reduced causing a retention of fat in this product.

Because the dry screening treatment in Set 3 was highly different from wet screening methods in the major components, Set 4 of ANOV was designed to involve only the three wet screening treatments and their final products including the low density fraction of air classification (Table 41).

F values for Set 4 of ANOV, presented in Table 17, demonstrate that the pH used in wet screening methods had a highly significant effect upon the fat, moisture, potassium, sodium, copper and zinc concentrations in the products. All components of the final products displayed differences ($P < .01$) indicating that the fractionation processes of wet screening, sieving and air classification had an effect on on the distribution in the final products of each component tested. The interaction between treatments and products was involved in phosphorus, copper and fluoride concentrations.

A set of orthogonal comparisons for Set 4 of ANOV demonstrated that within wet screening methods, the main variation was in fat content with pH 7 more effective than pH 10 and 10 more effective than 4 as an aid in the removal

Table 17

F Values for Set 4 of ANOV Involving Wet Screening Treatments and All their Final Products

ANOV for:	Treatment	Product	Prod x Treat	Residual
Protein	1.24	5.26**	0.30	24.9**
Fat	26.5 **	6.59**	1.44	34.7**
Moisture	9.02**	3.34*	0.60	98.9**
Ash	0.80	8.21**	0.45	62.9**
Phosphorus	0.00	130 **	3.21**	31.1**
Calcium	0.40	95.7 **	1.69	32.7**
Magnesium	3.45	15.1 **	0.60	11.5**
Potassium	46.4 **	5.4 **	0.09	26.4**
Sodium	27.0 **	24.6 **	0.14	43.9**
Copper	9.03**	15.93**	4.40**	16.6**
Manganese	1.50	15.27**	1.71	11.7**
Zinc	10.56**	4.87**	1.05	10.1**
Iron	0.30	34.88**	0.60	27.1**
Fluoride	0.98	76.70**	2.92*	16.0**
d.f.	2 and 30	4 and 30	8 and 30	30 and 45
$F_{.05}$	4.18	3.25	2.65	1.90
$F_{.01}$	5.39	4.02	3.17	2.14

* $P < .05$

** $P < .01$

Table 18

Sum of Squares of the Orthogonal Comparisons Involving the Wet Screening Treatments and all their Final Products

Comparisons	d.f.	S.S. Protein	S.S. Fat	S.S. Ash
Among treatments				
1. WS-7 vs WS-4, WS-10	1	2.99	9.65**	15.42
2. WS-4 vs WS-12	1	28.84	6.22**	0.31
Treatment sum of squares	2	31.83	15.87	15.73
Among Products				
1. C-12, C-20, C-35, F vs S	1	1734.49**	2.00	1896.17**
2. C-12, C-20, C-35 vs F	1	872.82**	1.70	760.69**
3. C-12, C20 vs C-35	1	316.56**	4.82	439.67**
4. C-12 vs C-20	1	18.06	2.38	5.05
Products sum of squares	4	2941.93	7.91	3101.57

** P .01

of fat. All products were significantly different from each other in protein and ash content with the exception of C-12 and C-20.

Differences in Mineral Content

Among treatments there was much variation in mineral composition; differences in potassium, sodium, copper, zinc and fluoride means were highly significant. Forty percent of the potassium and sodium was lost during extractions at pH 4 and 10 compared to 25% at pH 7, consequently potassium and sodium values for dry screening and wet screening at pH 7 were about 30% higher than those for wet screening at pH 4 or 10 in all the final products.

Treatments carried out at pH 7 also displayed higher concentrations of copper, zinc and iron in the final products. Copper means were much lower for wet screening at pH 10 than for the other three treatments. The products of wet screening at pH 4 had the highest concentrations of fluoride, because other elements were more soluble at this pH increasing the relative concentration of fluoride; less fluoride was removed by air classification for this extraction method.

Among the final products highly significant differences were found for the means of all the elements analyzed. In the low density fraction of air classification, having the highest concentration of ash, calcium, phosphorus, potassium and sodium were greatly concentrated ($P<.01$).

Iron, copper and zinc were more concentrated in the fine fraction where on the average values for iron were twice as large as in any other fraction. Fluoride and manganese displayed the greatest mean values for C-12 and C-20 where the bone matter had been collected.

Highly significant product x treatment interactions were found for phosphorus, calcium, copper and fluorine. The relative distribution of copper was not the same among the corresponding products of the treatments; both treatments at pH 7 and wet screening at pH 4 fine fractions had the highest concentration of copper among their products, but at pH 10, C-35 displayed the greater percentage of this element. Phosphorus, calcium and fluoride, associated with the bones and scales were more concentrated in the coarse fractions (C-12 and C-20) and the low density fraction than in C-35 and F. In the four treatments the ratio of calcium to phosphorus varied from about 1.6:1 for C-12 and C-20 to 1.3:1 for C-35 and F. These interactions indicate that the wet screening step tends to separate the protein fraction from the bone-scale fraction.

Total solids Mass Balance

PERCENT RECUPERATION: On the average the runs made at pH 7 had a higher percentage of protein recuperation (DS-7, 96.17%; WS-7, 95.11%) than the runs made at pH 10 (92.28%) and at pH 4 (90.82%). Yields obtained for the runs made at pH 7 are comparable to those reported for the

Halifax Process (Camberos, 1969a). Percent ash recuperation was also higher for DS-7 and WS-7 (94.03 and 94.48%) than for WS-4 and WS-10 (89.33 and 93.53%). More minerals and in larger amounts were soluble at pH 4 than at pH 7 or 10. Since protein and ash losses were proportional to each other, the percent composition remained about equal for the three wet screening methods, however, the total solids yield was 5% less for WS-4 and WS-10 than for WS-7.

More fat was extracted at pH 7 (DS-7, 99.48%; WS-7, 99.73%) than at pH 10 (98.58%) and at pH 4 (98.33%); this numerical difference of 1% was large enough in the sense that only the products extracted at pH 7 met the standard of less than 0.5% fat for FPC.

EFFECT OF AIR CLASSIFICATION: The low density fraction of air classification contained about 10% of the total solids but 15% of the total ash of WS-7, 12.5% of the total ash of WS-4 and 10.5% of the total of WS-10 was removed by this process.

The sum of the final fine product and C-35 was the only combination of fractions that met the standard of less than 100 ppm of fluoride and accounted for 38.49% of the total final product of WS-7, 34.49% of WS-4 and 31.78% of the total final solids of WS-10.

Solubility of FPC

Modification or adjustment of the solvent pH prior to extraction has been suggested as a means of increasing

Figure 7

Wet Screening pH-4, Run 1
Mass Balance of Solids: Protein, Ash and Fat

	Solids: grams	Protein grams	Ash grams	Fat grams
Fish	402.74:	227.58	50.37	124.79
Extraction				
for analyses →	37.40:	22.78	5.26	8.60
Wet Screening				
fines →	46.50:	39.85	4.75	0.25
Dry Screening				
Coarse 35 →	30.00:	23.66	3.75	0.12
Air Classification				
Coarse 12 →	77.00:	56.48	14.85	1.72
Coarse 20 →	47.50:	35.24	8.71	0.35
Low Density →	24.50:	16.93	6.23	0.01
Percent composition of total solids	100.00	76.35	16.98	1.09
Percent recuperation:		85.66	86.46	1.96
Percent extracted		14.34	13.56	98.04

Percent distribution of total final product solids:

Total	F	C-12	C-20	C-35	F
100%	20.62	34.15	21.06	13.30	10.87

Figure 8

Wet Screening pH-4, Run 2

Mass Balance of Solids: Protein, Ash and Fat

	Solids: grams	Protein grams	Ash grams	Fat grams
Fish	403.34:	215.44	49.66	139.04
Extraction				
for analyses →	36.00:	22.53	5.12	7.24
Wet Screening				
fines →	54.30:	45.91	5.09	0.60
Dry Screening				
Coarse 35 →	29.50:	24.29	3.51	0.39
Air Classification				
Coarse 12 →	77.00:	57.10	15.09	1.48
Coarse 20 →	47.50:	35.86	8.99	0.60
Low density →	24.50:	16.56	6.45	0.23
Percent composition of total solids	100.00	75.58	18.74	1.80
Percent recuperation		93.88	89.11	1.02
Percent extracted		6.12	10.89	98.98

Percent distribution of total final product solids:

Total	F	C-12	C-20	C-35	F
100%	23.33	33.08	20.40	12.67	10.52

Figure 9

Wet Screening pH-4, Run 3
Mass Balance of Solids: Protein, Ash and Fat

	Solids: grams	Protein grams	Ash grams	Fat grams
Fish ←	400.5:	215.27	49.32	148.57
Extraction				
for analyses →	42.80:	25.79	5.53	10.17
Wet Screening				
fines →	59.70:	57.12	7.09	1.13
Dry Screening				
Coarse 35 →	32.50:	25.68	4.17	0.57
Air Classification				
Coarse 12 →	51.00:	37.05	12.62	1.07
Coarse 20 →	53.50:	40.66	10.05	1.20
Low density →	21.00:	13.76	6.03	0.10
Percent composition of total solids	100.00	76.60	17.61	1.07
Percent Recuperation		92.93	92.44	2.03
Percent extracted		7.07	7.56	97.97

Percent distribution of total final product solids

Total	F	C-12	C-20	C-35	S
100%	31.20	22.05	24.00	14.35	9.40

Figure 10

Wet Screening pH-7, Run 1
Mass Balance of Solids: Protein, Ash and Fat

	Solids: grams	Protein grams	Ash grams	Fat grams
Fish	396.04:	213.64	48.24	134.16
Extraction				
for analyses →	32.00:	21.80	5.02	2.72
Wet Screening				
fines →	64.80:	55.30	6.30	0.14
Dry Screening				
Coarse 35 →	26.00:	20.96	3.49	0.07
Air Classification				
Coarse 12 →	72.70:	50.68	14.66	0.53
Coarse 20 →	40.40:	33.37	10.36	0.07
Low density →	22.60:	13.58	7.24	0.06
Percent composition of total solids	100.00	73.70	17.80	0.02
Percent recuperation		93.27	95.20	0.42
Percent extracted		6.73	4.80	99.58

Percent distribution of total final product solids

Total	F	C-12	C-20	C-35	S
100%	27.8	31.12	19.80	11.71	9.68

Figure 11

Wet Screening pH-7, Run 2

Mass Balance of Solids: Protein, Ash and Fat

	Solids: grams	Protein grams	Ash grams	Fat grams
Fish	390.75	212.68	47.62	123.62
Extraction				
for analyses →	34.57:	24.35	3.21	6.61
Wet Screening				
fines →	56.10:	47.35	5.81	0.06
Dry Screening				
Coarse 35 →	20.10:	15.50	3.81	0.00
Air Classification				
Coarse 12 →	86.00:	67.21	12.33	0.14
Coarse 20 →	42.70:	32.40	7.59	0.05
Low density →	23.00:	15.38	6.57	0.06
Percent composition of total solids	100.00	77.50	16.20	0.18
Percent recuperation		95.06	91.71	0.30
Percent extracted		4.94	9.29	99.70

Percent distribution of total final product solids

Total	F	C-12	C-20	C-35	S
100%	24.69	37.85	18.49	8.85	10.12

Figure 12

Wet Screening pH-7, Run 3

Mass Balance of Solids: Protein, Ash and Fat

	Solids: grams	Protein grams	Ash grams	Fat grams
Fish	400.19:	208.54	47.77	143.88
Extraction				
for analyses	41.10:	24.65	5.32	9.83
Wet Screening				
fines	49.00:	41.20	5.16	0.02
Dry Screening				
Coarse 35	23.00:	18.20	3.68	0.08
Air Classification				
Coarse 12	95.00:	71.50	17.81	0.04
Coarse 20	38.50:	29.25	7.23	0.01
Low density	27.00:	17.70	7.75	0.00
Percent composition of total solids	100.00	75.50	19.50	0.01
Percent recuperation		97.00	96.03	0.01
Percent extraction		3.00	3.92	99.99

Percent distribution of total final product solids

Total	F	C-12	C-20	C-35	S
100%	21.60	40.80	16.30	9.82	11.58

Figure 13

Wet Screening pH-10, Run 1
Mass Balance of Solids: Protein, Ash and Fat

	Solids: grams	Protein grams	Ash grams	Fat grams
Fish	393.48:	222.41	47.75	112.76
Extraction				
for analyses →	35.30:	22.00	4.96	7.38
Wet Screening				
fines →	51.00:	40.95	5.44	0.17
Dry Screening				
Coarse 35 →	17.00:	13.56	1.92	0.09
Air Classification				
Coarse 12 →	89.00:	63.89	18.79	0.59
Coarse 20 →	43.00:	30.98	9.35	0.20
Low density →	26.40:	18.63	5.57	0.08
Percent composition of total solids	100.00	74.21	18.14	0.46
Percent recuperation		92.93	96.40	0.92
Percent extracted		14.57	3.60	99.08

Percent distribution of total final product solids

Total	F	C-12	C-20	C-35	S
100%	22.53	39.29	18.99	7.51	11.66

Figure 14

Wet Screening pH-10, Run 2

Mass Balance of Solids: Protein, Ash and Fat

	Solids: grams	Protein grams	Ash grams	Fat grams
Fish	388.44:	216.54	47.88	139.70
Extraction				
for analyses →	42.40	23.96	5.47	13.42
Wet Screening				
fines →	42.00:	35.07	4.05	0.16
Dry Screening				
Coarse 35 →	28.00:	21.41	4.02	0.10
Air Classification				
Coarse 12 →	97.00:	67.95	17.06	1.08
Coarse 20 →	49.00:	36.09	8.48	0.15
Low density →	19.50:	13.01	4.83	0.16
Percent composition of total solids	100.00	73.69	16.33	0.70
Percent recuperation		91.18	91.71	1.18
Percent extracted		8.82	8.29	98.82

Percent distribution of total final product solids

Total	F	C-12	C-20	C-35	S
100%	20.62	34.15	21.06	13.30	10.87

Figure 15

Wet Screening pH-10, Run 3

Mass Balance of Solids: Protein, Ash and Fat

	Solids: grams	Protein grams	Ash grams	Fat grams
Fish →	368.50:	206.50	50.40	111.78
Extraction				
→ for analyses →	28.55:	16.24	4.60	5.60
Wet Screening				
→ fines →	44.20:	36.80	3.06	0.14
Dry Screening				
→ Coarse 35 →	32.50:	25.45	2.04	0.15
Air Classification				
→ Coarse 12 →	98.00:	73.16	17.61	1.73
→ Coarse 20 →	47.00:	35.70	8.90	0.41
→ Low density →	16.00:	10.21	5.03	0.08
Percent composition of total solids	100.00	78.00	15.70	1.08
Percent recuperation		92.75	92.52	2.17
Percent extracted		7.25	7.48	97.83

Percent distribution of total final product solids

Total	F	C-12	C-20	C-35	S
100%	18.62	42.02	19.85	13.75	6.74

Figure 16

Dry Screening pH-7, Run 1
Mass Balance of Solids: Protein, Ash and Fat

	Solids: grams	Protein grams	Ash grams	Fat grams
Fish ←	376.15:	204.43	47.30	124.42
↓ Extraction				
— for analyses →	33.70:	22.71	4.28	5.36
↓ Dry Screening				
— Coarse 12 →	46.20:	32.50	10.05	0.10
— Coarse 20 →	88.00:	61.16	19.42	0.36
— Coarse 35 →	39.00:	29.74	6.33	0.12
— Fine →	53.50:	44.67	4.73	0.25
Percent composition of total solids	100.00	74.14	17.89	0.37
Percent recuperation		93.63	94.78	0.67
Percent extracted		6.37	5.22	99.33

Percent distribution of total final product solids

Total	F	C-12	C-20	C-35
100%	23.60	20.38	38.62	17.35

Figure 17

Dry Screening pH-7, Run 2

Mass Balance of Solids: Protein, Ash and Fat

	Solids: grams	Protein grams	Ash grams	Fat grams
Fish	380.61:	207.63	51.09	121.89
Extraction				
for analyses	38.54:	24.62	5.53	6.76
Dry Screening				
Coarse 12	53.10:	38.98	10.47	0.12
Coarse 20	91.51:	64.79	20.08	0.19
Coarse 35	32.76:	24.70	5.94	0.07
Fine	57.62:	48.46	5.15	0.26
Percent composition of total solids	100.00	75.28	17.72	0.27
Percent recuperation		97.07	92.34	0.53
Percent extracted		2.89	7.66	99.42

Percent distribution of total final product solids

Total	F	C-12	C-20	C-35
100%	24.52	22.60	38.94	13.94

Figure 18

Dry Screening pH-7, Run 3
Mass Balance of Solids: Protein, Ash and Fat

	Solids: grams	Protein grams	Ash grams	Fat grams
Fish	379.00	201.81	45.57	122.00
Extraction				
for analyses	33.05:	22.37	5.46	3.75
Dry Screening				
Coarse 12	29.00	20.40	6.32	0.06
Coarse 20	85.50	59.42	18.87	0.36
Coarse 35	46.50	35.45	7.54	0.14
Fine	72.00	59.58	6.47	0.30
Percent composition of total solids	100.00	75.04	16.82	0.45
Percent recuperation		97.70	96.00	0.37
Percent extracted		2.30	2.00	99.63

Percent distribution of total final product solids

Total	F	C-12	C-20	C-35
100%	30.84	12.48	36.75	19.93

the poor solubility generally displayed by FPC.

In all cases, the pH of the water used for solubility tests had a more profound effect on the nitrogen which went into solution than did the treatment involved. These results are presented in Table 19 as mg of nitrogen soluble in 100 ml of water for each of the eight products tested at each adjusted pH 2 to 12; Table 20 presents the same data expressed as the percent nitrogen of each product which went into solution at each pH.

In general the nitrogen of the coarse fractions was more soluble than that of the fine fractions, with the exception of WS-10 fine fraction which was more soluble than WS-7 coarse fraction. WS-7 and DS-7 coarse products showed the higher soluble nitrogen values, but among fine fractions WS-10 was more soluble than the other three fine products. Products of WS-4 consistently displayed the poorest solubility results.

The pH of the water used for solubility tests upon the products showed a general pattern of decreased nitrogen solubility as the pH rose from 2 to 6, a slight increase was displayed at pH 7 and the solubility again dropped as the solutions become more basic up to pH 9. A slight rise was noted at pH 10, but the greatest solubility for all products, both coarse and fine was displayed at pH 11 and 12.

Table 19

Milligrams of Nitrogen of a 1g Sample Soluble in 100 ml Water at Different pH

pH	Fine Fraction				Coarse Fraction			
	DS-7	WS-4	WS-7	WS-10	DS-7	WS-4	WS-7	WS-10
2	14.0	12.0	14.6	17.4	18.2	14.6	17.9	15.1
3	12.3	9.0	12.3	13.4	13.7	11.8	15.7	10.9
4	10.9	7.8	11.2	13.2	14.0	9.2	14.0	9.8
5	12.3	7.6	10.0	12.0	14.3	9.5	12.9	9.2
6	10.9	9.2	14.0	14.3	14.0	13.2	17.9	13.4
7	15.7	13.4	19.3	20.8	25.9	20.7	28.0	22.1
8	10.9	9.0	12.6	11.8	12.3	8.4	11.2	8.4
9	10.9	9.0	12.0	14.3	12.3	9.5	13.2	10.6
10	12.9	12.6	14.0	14.3	13.7	9.0	14.8	11.5
11	21.0	20.2	27.2	26.9	27.7	23.2	20.4	19.0
12	39.8	31.4	47.0	45.4	41.4	32.8	42.0	34.7

Table 20

Percent Nitrogen of a 1g Sample Soluble
in 100 ml Water at Defferent pH

pH	Fine Fraction				Coarse Fraction			
	DS-7	WS-4	WS-7	WS-10	DS-7	WS-4	WS-7	WS-10
2	10.9	9.1	10.8	13.0	15.4	13.7	15.2	13.0
3	9.6	6.7	9.1	10.1	11.6	11.1	13.3	9.4
4	8.5	5.9	8.3	9.9	11.8	8.7	11.9	8.4
5	9.6	5.7	7.9	9.0	12.1	9.0	10.9	7.9
6	8.9	7.0	10.4	10.7	11.8	12.4	15.2	11.5
7	12.2	10.1	14.3	15.5	21.1	19.5	23.8	19.0
8	8.5	6.7	9.3	8.8	10.4	7.9	9.5	7.2
9	8.5	6.7	8.9	10.7	10.4	9.0	11.2	9.1
10	10.0	9.5	10.4	10.7	11.6	8.4	12.6	9.9
11	16.3	15.1	20.1	20.1	23.4	21.9	17.4	16.4
12	30.9	23.6	34.9	34.0	35.0	30.8	35.7	29.8

SUMMARY AND CONCLUSIONS

From the results of this investigation, it is possible to evaluate the modifications made to the Bureau of Commercial Fisheries method for fish protein concentrate (FPC) production.

Azeotropic isopropyl alcohol (AIPA) at 65°C was somewhat less efficient than extraction at 78°C with 91% isopropyl alcohol (BCF, 1966; Loustaunau, 1971), however, the final products of the treatments made at pH 7, dry screening at pH 7 (DS-7) and wet screening at pH 7 (WS-7), met the FDA standard of less than 0.5% fat in FPC. The use of AIPA is recommended as a more economical and feasible solvent concentration for FPC production. A higher temperature, 78°C instead of 65°C, would increase fat extraction efficiency.

Passing the distillate of the first vacuum distillation of used solvent through a cation exchange resin is recommended as a means of insuring the recuperation of the azeotrope of isopropyl alcohol during the second distillation.

The adjustment of solvent pH prior to extraction is not only unnecessary, but reduces protein yield, fat extraction efficiency and yield of the final fine product in addition to extracting more potassium, which is a valuable nutrient. Acid pH tends to increase the relative concentration of fluoride by solubilizing other components of the

ash.

Nitrogen of WS-10 final fine product was no more soluble than nitrogen from the fine products extracted at pH 7, and solubility values of WS-4 products were reduced by this treatment. From the results of the solubility data, it is recommended that further treatment of the final products obtained be used to modify the solubility properties rather than attempting to modify the extraction process in order to increase the solubility of the final products.

Dry screening of the final product is not necessary, providing that the wet screening modification is adapted as part of each of the three extractions, which would increase the efficiency of separation of the fine fraction. No significant differences were found between the fractions C-12 and C-20 of the dry screening operation.

Wet screening proved to be effective in separating the low ash, low fluoride fraction (82% protein, 10% ash and less than 50 ppm of fluoride) from the high bone-scale fraction (70% protein, 20% ash and more than 100 ppm of fluoride); it is suggested that this refining step be optimized for mesh size of the extraction unit, agitation technique, loading the extraction unit to an optimal capacity, ratio of solvent to solids, extraction temperature and particle size of the feeding product.

Air classification of the final coarse product proved to be a definite improvement by removing up to 15% of the total ash from the coarse products while removing only 10%

of the total solids.

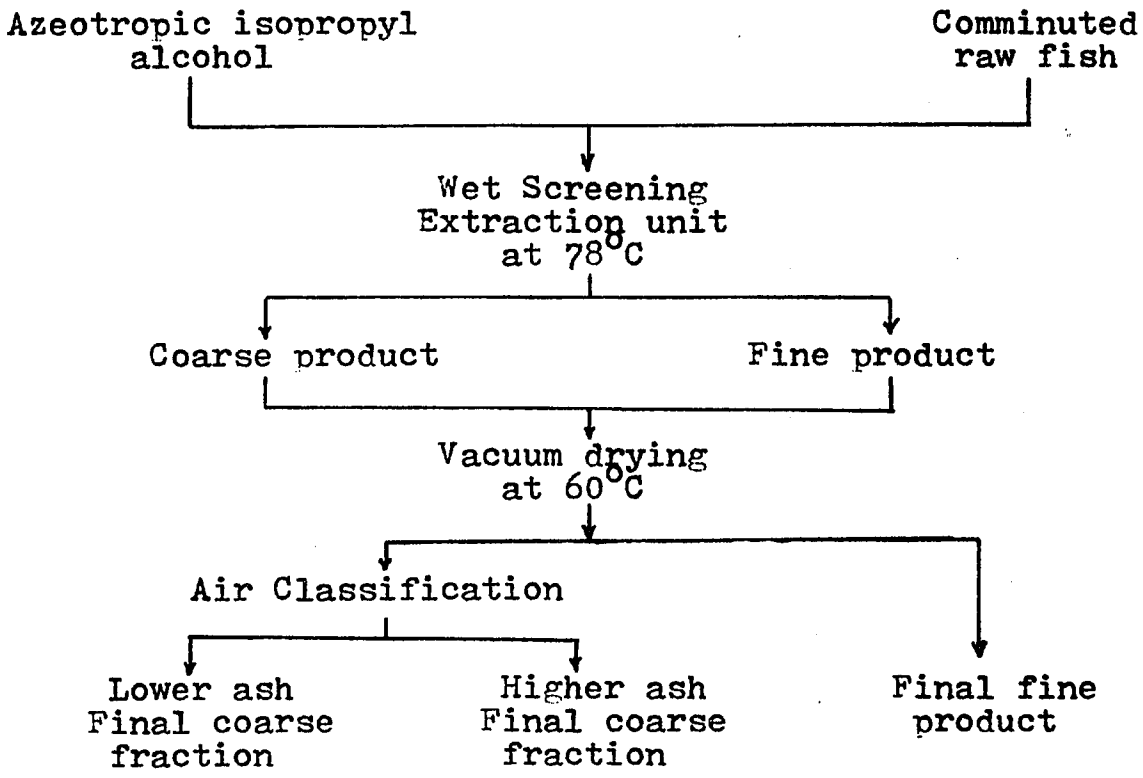
As a conslusion to this study, a modified process for FPC production from whole fish has been developed. In this process, fat and water from comminuted whole fish is extracted with azeotropic isopropyl alcohol (pH 7 ± 0.2) at 78°C in an extraction unit with a screening device that separates the fine fraction that passes through the screen from the coarse fraction retained in the screen. The dry and desolventized final fraction meets the standard of FPC for human consumption and has an added nutritive value from its mineral components.

Separation of the different bulk density fractions of the coarse fraction would increase the yield of FPC and the remaining product, a high quality deodorized fish meal, could be used in animal and poultry feeds. The solvent can be recuperated by double distillation from the filtrate and purified by passing the distillate through a cation exchange resin prior to the second distillation.

For producing useful products and byproducts, this process would eliminate waste and waste disposal problems and would reduce the overall cost of the process in addition to allowing the use of fish independently of its size (Fig. 19).

Figure 19

Proposed Method for FPC Production from Whole Fish



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APPENDIX

Table 21

Dry Screening pH-7
Protein and Fat Composition of the Products of Three Runs

Products	Protein (%) Run 1	Run 2	Run 3	Fat (%) Run 1	Run 2	Run 3
Raw Product- wet basis	16.35	16.61	16.44	9.95	9.75	9.76
Solids of raw product	52.20 52.30	53.10 52.60	53.10 53.40	31.00 32.60	31.34 30.71	32.27 32.11
Solids of first extraction	54.20 54.60	57.50 58.40	61.80 62.30	30.00 30.30	26.30 26.10	16.89 17.00
Solids of second extraction	73.10 73.30	73.50 73.60	74.10 73.90	3.13 3.65	3.83 3.52	4.20 4.06
Final coarse fraction retained by sieve # 12	70.10 70.60	73.90 72.90	68.20 68.30	0.18 0.24	0.30 0.16	0.62 0.42
Final coarse fraction retained by sieve # 20	69.50 69.50	70.80 70.80	69.30 69.20	0.38 0.44	0.23 0.18	0.35 0.36
Final coarse fraction retained by sieve # 35	76.56 75.91	75.60 75.15	75.82 76.90	0.28 0.31	0.22 0.22	0.07 0.15
Final fine fraction	83.99 83.00	84.30 83.92	82.75 82.70	0.48 0.56	0.44 0.46	0.44 0.39

Table 22

Dry Screening pH-7
Moisture and Ash Composition of the Products of Three Runs

Products	Moisture (%) Run 1	Run 2	Run 3	Ash (%) Run 1	Run 2	Run 3
Raw Product- wet basis	70.18	69.90	70.36	3.78	4.09	3.65
Solids of raw product	4.96 4.52	4.18 4.26	2.18 2.27	12.14 12.04	13.92 12.09	12.19 11.86
Solids of first extraction	4.67 4.71	4.98 5.00	3.45 3.44	11.85 11.87	11.87 11.91	15.95 16.05
Solids of second extraction	7.63 7.58	5.13 5.11	3.64 3.72	16.15 15.81	18.10 18.78	17.28 17.10
Final coarse fraction retained by sieve # 12	7.90 7.94	7.87 7.90	8.96 8.96	22.04 21.56	19.71 19.73	24.10 23.68
Final coarse fraction retained by sieve # 20	8.49 8.48	7.97 8.09	9.71 9.83	22.03 22.11	22.02 21.88	21.47 21.75
Final coarse fraction retained by sieve # 35	7.67 7.64	7.41 7.40	7.97 7.91	16.19 16.25	17.79 18.46	15.69 15.59
Final fine fraction	7.63 7.62	7.04 7.06	8.27 8.29	8.81 8.89	8.96 8.93	9.01 8.96

Table 23

Wet Screening pH-4
Protein and Fat Composition of the Products of Three Runs

Products	Protein (%) Run 1	Run 2	Run 3	Fat (%) Run 1	Run 2	Run 3
Raw Product- wet basis	17.92	17.23	17.22	9.83	11.12	11.88
Solids of raw product	54.60	52.00	52.00	29.47	33.40	36.15
	54.60	52.90	52.50	30.40	34.30	35.98
Solids of first extraction	59.00	57.00	57.00	27.92	28.29	27.97
	56.50	57.40	57.50	28.36	28.24	28.91
Solids of second extraction	65.40	70.60	63.50	14.79	8.21	18.50
	66.60	70.30	64.00	14.73	8.12	18.10
Final coarse fraction retained by sieve # 12	73.30	74.10	72.80	2.30	1.97	2.11
	73.40	74.20	72.50	2.18	1.88	2.10
Final coarse fraction retained by sieve # 20	75.00	75.80	75.40	0.81	1.24	1.98
	73.40	75.20	76.60	0.68	1.28	2.51
Final coarse fraction retained by sieve # 35	78.80	81.80	79.20	0.36	1.30	1.74
	78.90	82.90	78.80	0.46	1.32	1.77
Final fine fraction of wet screening	86.00	84.30	81.60	0.51	1.22	1.51
	85.40	84.80	82.30	0.58	1.01	1.72
Low density fraction of air classification	68.90	66.40	64.50	0.01	1.00	0.44
	69.30	68.80	66.60	0.01	0.89	0.52

Table 24

Wet Screening pH-4
Moisture and Ash Composition of the Products of Three Runs

Products	Moisture (%) Run 1	Run 2	Run 3	Ash (%) Run 1	Run 2	Run 3
Raw Product- wet basis	67.73	67.75	67.96	3.97	3.97	3.94
Solids of raw product	1.71 1.63	1.81 1.85	2.78 2.80	12.16 12.01	11.83 12.35	11.90 12.04
Solids of first extraction	1.55 1.46	2.36 2.38	3.23 3.14	13.07 13.43	12.58 12.71	12.75 12.91
Solids of second extraction	2.68 2.85	4.81 4.84	5.07 5.90	15.46 15.20	16.37 16.63	13.11 12.97
Final coarse fraction retained by sieve # 12	6.53 6.63	5.24 5.25	4.71 5.24	19.54 19.03	19.51 19.69	24.30 25.20
Final coarse fraction retained by sieve # 20	6.35 6.60	5.32 5.39	5.47 5.53	18.15 18.54	18.89 18.98	18.75 18.81
Final coarse fraction retained by sieve # 35	7.00 7.03	4.33 4.47	4.74 4.35	12.44 12.58	12.35 11.44	12.84 12.84
Final fine fraction of wet screening	4.12 4.01	2.38 2.37	2.87 3.06	10.16 10.27	9.30 9.45	10.12 10.23
Low density fraction of air classification	7.53 7.51	5.57 5.62	5.64 5.61	25.87 24.98	26.48 26.17	28.70 28.70

Table 25

Wet Screening pH-7
Protein and Fat Composition of the Products of Three Runs

Products	Protein (%) Run 1	Run 2	Run 3	Fat (%) Run 1	Run 2	Run 3
Raw Product- wet basis	17.09	17.02	16.68	10.72	9.81	11.51
Solids of raw product	54.30 53.30	55.50 53.00	53.80 54.40	33.70 33.80	30.11 31.44	31.60 31.51
Solids of first extraction	60.80 60.00	64.30 63.40	65.10 65.90	22.30 24.00	20.67 20.52	18.36 17.89
Solids of second extraction	77.60 79.30	77.00 76.40	76.50 74.90	4.20 3.80	0.85 1.07	3.71 3.80
Final coarse fraction retained by sieve # 12	70.10 69.30	77.50 78.80	75.40 75.90	0.84 0.78	0.27 0.08	0.44 0.36
Final coarse fraction retained by sieve # 20	71.30 71.80	76.80 77.50	76.00 75.60	0.12 0.22	0.08 0.18	0.31 0.44
Final coarse fraction retained by sieve # 35	80.00 81.20	77.50 76.80	79.60 78.90	0.32 0.39	0.01 0.02	0.18 0.21
Final fine fraction of wet screening	86.10 84.60	84.00 84.80	84.40 84.10	0.22 0.23	0.16 0.08	0.11 0.01
Low density fraction of air classification	58.90 61.30	66.80 66.90	70.00 69.90	0.27 0.29	0.14 0.25	0.01 0.01

Table 26

Wet Screening pH-7
Moisture and Ash Composition of the Products of Three Runs

Products	Moisture (%) Run 1	Run 2	Run 3	Ash (%) Run 1	Run 2	Run 3
Raw Product- wet basis	68.41	68.74	68.97	3.78	3.81	3.82
Solids of raw product	1.66 1.77	2.72 2.75	2.07 2.21	12.10 11.67	12.63 12.72	11.96 12.52
Solids of first extraction	5.93 5.91	3.27 3.30	2.22 2.41	12.90 12.90	15.61 14.90	15.50 14.66
Solids of second extraction	4.03 3.72	3.77 3.77	2.71 2.67	15.63 16.01	17.15 17.11	16.98 16.99
Final coarse fraction retained by sieve # 12	7.56 7.06	6.18 6.37	6.26 6.08	22.18 22.17	14.27 14.40	18.57 18.63
Final coarse fraction retained by sieve # 20	6.62 6.30	4.48 4.64	6.05 6.08	22.58 22.37	18.00 18.16	18.74 18.66
Final coarse fraction retained by sieve # 35	6.73 6.32	4.14 4.32	6.22 6.30	13.91 13.69	18.47 19.46	16.06 16.04
Final fine fraction of wet screening	6.32 6.35	2.45 2.68	6.58 6.56	9.79 9.67	10.17 10.57	10.61 10.50
Low density fraction of air classification	8.30 8.25	4.40 4.43	6.15 6.15	31.97 32.13	28.53 28.57	24.72 24.90

Table 27

Wet Screening pH-10
Protein and Fat Composition of the Products of Three Runs

Products	Protein (%) Run 1	Run 2	Run 3	Fat (%) Run 1	Run 2	Run 3
Raw Product- wet basis	17.79	17.33	16.52	9.02	11.18	8.94
Solids of raw product	56.90 56.00	52.40 52.50	54.80 56.00	28.38 28.86	33.83 33.83	26.48 26.78
Solids of first extraction	57.80 60.00	52.80 53.30	59.90 60.20	25.70 25.95	29.44 29.43	21.33 21.74
Solids of second extraction	67.90 66.30	60.90 61.50	61.00 61.60	14.14 14.07	18.07 17.70	17.65 16.85
Final coarse fraction retained by sieve # 12	70.50 71.00	70.80 69.30	74.80 74.50	0.62 0.60	1.06 1.17	1.80 1.74
Final coarse fraction retained by sieve # 20	72.50 71.60	72.50 74.80	75.90 76.00	0.51 0.44	0.34 0.27	0.85 0.89
Final coarse fraction retained by sieve # 35	79.50 80.00	76.50 76.40	78.30 78.30	0.59 0.42	0.26 0.46	0.62 0.32
Final fine fraction of wet screening	81.10 79.50	83.50 83.50	83.00 83.50	0.31 0.37	0.36 0.41	0.14 0.17
Low density fraction of air classification	70.80 70.30	66.80 66.60	64.50 63.10	0.32 0.32	0.76 0.87	0.55 0.45

Table 28

Wet Screening pH-10
Moisture and Ash Composition of the Products of Three Runs

Products	Moisture (%) Run 1	Run 2	Run 3	Ash (%) Run 1	Run 2	Run 3
Raw product- wet basis	69.16	68.92	70.51	3.82	3.83	4.03
Solids of raw product	2.15 2.17	5.93 5.94	5.55 5.63	12.01 12.26	11.70 11.49	13.58 13.46
Solids of first extraction	2.76 2.56	5.81 5.88	5.53 5.87	13.10 12.90	11.90 12.07	13.92 13.75
Solids of second extraction	2.10 2.10	7.61 7.61	5.25 5.12	15.43 15.49	14.15 14.18	14.72 14.83
Final coarse fraction retained by sieve # 12	6.61 6.26	7.95 8.23	6.89 6.75	21.65 20.57	18.43 16.74	18.01 17.93
Final coarse fraction retained by sieve # 20	5.69 5.80	7.76 7.82	5.77 5.63	21.93 21.58	17.32 17.28	18.57 19.30
Final coarse fraction retained by sieve # 35	7.87 7.89	7.83 7.85	6.26 6.28	11.20 11.41	14.12 14.15	14.42 16.24
Final fine fraction of wet screening	6.04 5.93	6.54 5.93	6.54 7.32	10.43 10.90	9.67 9.61	10.15 10.31
Low density fraction of air classification	8.43 8.31	8.30 8.16	6.96 7.04	22.17 23.46	24.80 24.97	31.44 31.44

Table 29

Mineral Composition of the Products of
Dry Screening pH-7, Run One

Products	P %	Ca %	Mg %	K %	Na %	Cu ppm	Mn ppm	Zn ppm	Fe ppm	F ppm
Raw Product- wet basis	0.73	1.10	0.04	0.28	0.14	2.2	5.2	9.7	57.9	25.5
Solids of raw product	2.32	3.62	0.14	0.87	0.51	6	16	30	180	81
	2.33	3.45	0.14	0.91	0.39	8	17	32	190	82
Solids of first extraction	2.16	3.33	0.14	0.60	0.33	8	28	93	210	-
	2.12	4.18	0.13	0.57	0.34	7	26	93	210	-
Solids of second extraction	3.07	4.58	0.18	0.72	0.45	8	28	93	210	-
	3.13	4.78	0.18	0.72	0.45	9	26	93	210	-
Final coarse fraction retained by sieve # 12	4.02	6.67	0.20	0.77	0.53	10	26	71	170	196
	4.07	6.75	0.19	0.77	0.54	12	29	67	160	225
Final coarse fraction retained by sieve # 20	4.26	6.15	0.20	0.79	0.56	16	22	85	130	196
	4.25	6.30	0.20	0.79	0.54	19	25	82	130	205
Final coarse fraction retained by sieve # 35	2.95	4.12	0.18	0.73	0.47	22	19	102	186	128
	3.37	4.61	0.19	0.72	0.50	21	21	98	172	132
Final fine fraction	1.28	1.57	0.19	0.73	0.37	42	14	110	428	31
	1.28	1.53	0.19	0.72	0.37	47	14	115	450	34

Table 30

Mineral Composition of the Products of
Dry Screening pH-7, Run Two

Products	P %	Ca %	Mg %	K %	Na %	Cu ppm	Mn ppm	Zn ppm	Fe ppm	F ppm
Raw Product- wet basis	0.66	0.95	0.04	0.26	0.15	2.0	6.1	11.6	55.0	28.3
Solids of raw product	2.05	2.94	0.11	0.82	0.43	6	20	37	170	93
	2.14	3.08	0.12	0.86	0.52	7	19	37	180	87
Solids of first extraction	2.24	3.28	0.14	0.65	0.34	12	18	69	220	-
	2.22	3.16	0.14	0.62	0.34	19	17	65	170	-
Solids of second extraction	3.75	6.23	0.19	0.77	0.49	13	22	110	190	-
	3.63	5.89	0.20	0.79	0.51	13	25	97	220	-
Final coarse fraction retained by sieve # 12	3.78	5.80	0.20	0.77	0.51	26	17	92	130	213
	3.69	5.54	0.19	0.79	0.49	26	19	92	120	207
Final coarse fraction retained by sieve # 20	4.25	6.52	0.20	0.87	0.55	22	24	90	140	161
	4.25	6.39	0.20	0.82	0.54	21	22	82	130	187
Final coarse fraction retained by sieve # 35	3.34	4.73	0.18	0.78	0.50	23	18	97	167	158
	3.46	4.95	0.19	0.77	0.50	25	19	95	160	138
Final fine fraction	1.43	1.51	0.18	0.69	0.37	48	15	114	506	42
	1.44	1.48	0.18	0.68	0.37	46	13	117	501	35

Table 31

Mineral Composition of the Products of
Dry Screening pH-7, Run Three

Products	P %	Ca %	Mg %	K %	Na %	Cu ppm	Mn ppm	Zn ppm	Fe ppm	F ppm
Raw Product- wet basis	0.64	0.91	0.04	0.28	0.13	3.0	5.9	11.0	53.2	25.5
Solids of raw product	2.17	3.08	0.12	0.91	0.46	10	22	37	160	83
	2.03	2.90	0.12	0.91	0.41	10	17	35	180	80
Solids of first extraction	2.97	5.00	0.16	0.72	0.42	20	22	64	170	-
	2.90	4.60	0.17	0.72	0.39	21	24	67	170	-
Solids of second extraction	3.33	5.09	0.20	0.72	0.43	15	26	67	200	-
	3.26	5.02	0.19	0.72	0.43	15	24	67	190	-
Final coarse fraction retained by sieve # 12	4.40	7.02	0.19	0.72	0.52	24	30	100	160	236
	4.54	7.20	0.19	0.69	0.52	23	30	98	140	255
Final coarse fraction retained by sieve # 20	4.27	6.22	0.20	0.72	0.52	28	30	80	170	208
	4.24	6.39	0.20	0.72	0.47	26	34	90	190	181
Final coarse fraction retained by sieve # 35	2.88	3.64	0.18	0.56	0.38	25	24	87	187	133
	2.58	4.12	0.17	0.62	0.41	23	24	89	196	125
Final fine fraction	1.42	1.52	0.16	0.58	0.33	44	18	129	423	38
	1.36	1.43	0.17	0.59	0.33	41	18	130	436	40

Table 32

Mineral Composition of the Products of
Wet Screening pH-4, Run One

Products	P %	Ca %	Mg %	K %	Na %	Cu ppm	Mn ppm	Zn ppm	Fe ppm	F ppm
Raw Product- wet basis	0.73	1.12	0.04	0.30	0.12	1.8	4.8	14	45	25
Solids of raw product	2.32	3.48	0.12	0.94	0.37	5	14	37	140	75
	2.23	3.48	0.12	0.92	0.36	6	16	50	140	82
Solids of first extraction	2.54	4.00	0.13	0.54	0.24	6	20	37	140	-
	2.49	3.71	0.12	0.52	0.23	5	20	37	150	-
Solids of second extraction	2.96	4.35	0.15	0.52	0.24	7	22	42	180	-
	3.02	4.68	0.15	0.52	0.24	9	22	44	180	-
Final coarse fraction retained by sieve # 12	3.76	5.48	0.17	0.52	0.26	14	26	47	150	281
	3.79	5.72	0.16	0.52	0.26	15	25	53	130	277
Final coarse fraction retained by sieve # 20	3.50	5.30	0.17	0.52	0.27	15	25	90	140	254
	3.47	5.08	0.17	0.52	0.26	18	24	80	140	261
Final coarse fraction retained by sieve # 35	2.53	3.71	0.15	0.47	0.22	12	15	60	180	117
	2.52	3.71	0.15	0.48	0.23	13	19	80	150	139
Final fine fraction of wet screening	1.97	2.74	0.19	0.52	0.21	51	19	70	490	47
	1.98	2.78	0.20	0.44	0.20	42	19	90	480	43
Low density fraction of air classification	5.34	6.32	0.21	0.64	0.36	27	29	54	140	178
	5.38	6.69	0.21	0.64	0.36	26	31	53	140	165

Table 33

Mineral Composition of the Products of
Wet Screening pH-4, Run Two

Products	P %	Ca %	Mg %	K %	Na %	Cu ppm	Mn ppm	Zn ppm	Fe ppm	F ppm
Raw Product- wet basis	0.73	1.03	0.04	0.28	0.11	2.1	5.5	15	42	29
Solids of raw product	2.21	3.28	0.11	0.87	0.33	8	17	38	130	83
	2.17	3.11	0.11	0.89	0.34	5	17	53	130	97
Solids of first extraction	2.42	3.48	0.14	0.44	0.22	8	17	45	150	-
	2.43	3.56	0.14	0.44	0.22	10	15	44	150	-
Solids of second extraction	3.22	4.67	0.18	0.44	0.25	8	21	57	180	-
	3.33	4.72	0.19	0.46	0.25	9	19	52	180	-
Final coarse fraction retained by sieve # 12	3.75	5.95	0.20	0.38	0.23	12	28	64	190	296
	3.72	5.92	0.21	0.38	0.23	13	28	61	180	338
Final coarse fraction retained by sieve # 20	3.57	5.37	0.21	0.36	0.23	12	25	56	190	244
	3.64	5.68	0.20	0.36	0.23	17	25	55	190	243
Final coarse fraction retained by sieve # 35	2.46	3.48	0.18	0.34	0.19	16	22	72	210	126
	2.43	3.37	0.18	0.32	0.19	17	18	78	220	109
Final fine fraction of wet screening	1.87	3.53	0.19	0.32	0.17	33	19	72	420	47
	1.85	3.61	0.18	0.34	0.17	31	17	72	450	49
Low density fraction of air classification	5.34	7.47	0.23	0.52	0.37	24	30	63	180	166
	5.34	7.47	0.24	0.54	0.38	24	29	59	180	176

Table 34

Mineral Composition of the Products of
Wet Screening pH-4, Run Three

Products	P %	Ca %	Mg %	K %	Na %	Cu ppm	Mn ppm	Zn ppm	Fe ppm	F ppm
Raw Product- wet basis	0.68	0.99	0.04	0.25	0.11	2.4	4.7	11	37	24
Solids of raw product	2.12	2.90	0.12	0.76	0.33	6	14	34	110	77
	2.10	3.27	0.12	0.78	0.34	9	13	35	120	73
Solids of first extraction	2.27	3.24	0.13	0.40	0.21	7	15	41	160	-
	2.22	3.23	0.13	0.40	0.21	6	15	38	150	-
Solids of second extraction	2.54	3.80	0.15	0.36	0.19	10	16	43	170	-
	2.42	3.80	0.15	0.32	0.19	10	18	45	160	-
Final coarse product retained by sieve # 12	3.82	6.20	0.19	0.34	0.24	18	26	59	140	404
	3.80	6.30	0.19	0.34	0.24	17	28	50	140	433
Final coarse product retained by sieve # 20	3.57	5.52	0.19	0.32	0.22	16	25	53	150	273
	3.50	5.52	0.19	0.32	0.23	15	26	48	140	280
Final coarse product retained by sieve # 35	2.57	3.58	0.17	0.30	0.19	18	17	65	160	141
	2.47	3.43	0.16	0.28	0.18	16	17	65	190	139
Final fine fraction of wet screening	1.54	2.40	0.19	0.32	0.17	25	19	72	380	52
	1.92	2.54	0.20	0.32	0.16	25	18	72	400	52
Low density fraction of air classification	5.90	7.64	0.27	0.52	0.38	30	32	63	150	205
	5.60	7.72	0.29	0.52	0.38	29	33	63	150	193

Table 35

Mineral Composition of the Products of
Wet Screening pH-7, Run One

Products	P %	Ca %	Mg %	K %	Na %	Cu ppm	Mn ppm	Zn ppm	Fe ppm	F ppm
Raw Product- wet basis	0.69	1.00	0.04	0.28	0.12	2.2	4.6	8.5	58.4	27.6
Solids of raw product	2.22	3.14	0.13	0.89	0.38	6	14	27	190	87
	2.12	3.19	0.13	0.89	0.36	8	15	27	180	88
Solids of first extraction	2.47	3.78	0.16	0.56	0.25	8	20	38	200	-
	2.43	3.67	0.15	0.58	0.25	7	18	39	200	-
Solids of second extraction	3.17	4.72	0.19	0.67	0.34	11	23	51	240	-
	3.20	4.79	0.20	0.65	0.33	10	24	51	250	-
Final coarse fraction retained by sieve # 12	3.63	6.04	0.18	0.68	0.34	20	20	78	190	277
	3.61	5.92	0.18	0.62	0.36	23	23	66	210	266
Final coarse fraction retained by sieve # 20	3.16	4.78	0.18	0.53	0.34	17	31	66	170	296
	3.22	4.89	0.19	0.54	0.35	16	32	66	160	297
Final coarse fraction retained by sieve # 35	2.81	3.85	0.18	0.52	0.27	19	22	68	170	129
	2.87	4.29	0.17	0.50	0.28	16	19	66	160	134
Final fine fraction of wet screening	1.99	2.56	0.20	0.55	0.27	44	18	86	524	52
	1.95	2.60	0.20	0.53	0.27	41	16	85	504	47
Low density fraction of air classification	6.40	8.43	0.23	0.64	0.38	27	29	63	155	228
	6.49	8.39	0.24	0.65	0.37	29	31	59	150	223

Table 36

Mineral Composition of the Products of
Wet Screening pH-7, Run Two

Products	P %	Ca %	Mg %	K %	Na %	Cu ppm	Mn ppm	Zn ppm	Fe ppm	F ppm
Raw Product- wet basis	0.71	1.10	0.03	0.29	0.12	2.0	5.2	10.2	50	28.8
Solids of raw product	2.31	3.58	0.11	0.94	0.38	7	16	35	160	93
	2.26	3.43	0.11	0.92	0.36	6	17	30	160	91
Solids of first extraction	2.64	4.09	0.14	0.77	0.33	10	16	40	190	-
	2.83	4.19	0.16	0.78	0.34	10	16	38	200	-
Solids of second extraction	3.32	4.50	0.17	0.84	0.37	7	22	71	190	-
	3.23	4.64	0.17	0.86	0.38	8	22	67	210	-
Final coarse fraction retained by sieve # 12	2.75	4.09	0.17	0.71	0.32	21	21	62	230	204
	2.41	3.76	0.14	0.64	0.30	18	22	77	210	181
Final coarse fraction retained by sieve # 20	3.34	4.72	0.18	0.72	0.34	17	22	68	160	239
	3.35	4.92	0.20	0.57	0.33	15	23	72	160	245
Final coarse fraction retained by sieve # 35	3.49	4.88	0.18	0.77	0.35	15	22	70	190	161
	3.50	5.19	0.15	0.76	0.35	16	21	73	170	159
Final fine fraction of wet screening	1.40	3.20	0.18	0.73	0.32	56	20	132	380	44
	1.37	3.06	0.20	0.72	0.32	52	21	141	360	45
Low density fraction of air classification	5.66	7.19	0.22	0.89	0.49	22	30	87	140	213
	5.72	7.32	0.22	0.98	0.46	21	32	96	140	224

Table 37

Mineral Composition of the Products of
Wet Screening pH-7, Run Three

Products	P %	Ca %	Mg %	K %	Na %	Cu %	Mn ppm	Zn ppm	Fe ppm	F ppm
Raw Product- wet basis	0.73	1.07	0.03	0.29	0.11	2.8	4.2	14.0	51.2	24.7
Solids of raw product	2.32	3.48	0.12	0.94	0.35	10	13	48	160	78
	2.27	3.43	0.11	0.94	0.35	8	14	42	170	81
Solids of first extraction	2.35	4.00	0.14	0.73	0.31	10	18	61	170	-
	2.36	4.00	0.14	0.74	0.31	12	20	63	180	-
Solids of second extraction	3.35	4.52	0.17	0.77	0.36	11	24	58	261	-
	3.27	4.24	0.18	0.84	0.36	12	22	72	240	-
Final coarse fraction retained by sieve # 12	3.50	5.19	0.17	0.77	0.34	20	22	87	180	213
	3.48	5.10	0.18	0.75	0.35	22	22	90	190	192
Final coarse fraction retained by sieve # 20	3.47	5.20	0.17	0.74	0.34	24	25	58	180	253
	3.58	5.00	0.17	0.74	0.35	21	24	68	180	237
Final coarse fraction retained by sieve # 35	2.94	3.94	0.17	0.74	0.34	13	19	68	210	199
	2.94	4.00	0.17	0.72	0.32	17	20	62	240	173
Final fine fraction of wet screening	2.00	2.74	0.18	0.73	0.32	40	21	78	594	42
	1.98	2.78	0.18	0.78	0.30	41	22	82	509	44
Low density fraction of air classification	5.06	6.48	0.18	0.83	0.46	35	25	69	170	176
	5.07	6.40	0.19	0.83	0.45	34	22	67	150	170

Table 38

Mineral Composition of the Products of
Wet Screening pH-10, Run One

Products	P %	Ca %	Mg %	K %	Na %	Cu ppm	Mn ppm	Zn ppm	Fe ppm	F ppm
Raw Product- wet basis	0.74	0.05	0.04	0.27	0.13	3.4	4.9	12	43.2	24
Solids of raw product	2.50	3.48	0.14	0.88	0.41	12	18	42	130	80
	2.30	3.33	0.13	0.84	0.40	10	14	38	150	78
Solids of first extraction	3.07	4.42	0.17	0.40	0.24	6	23	41	180	-
	3.06	4.72	0.17	0.38	0.24	6	21	46	190	-
Solids of second extraction	1.51	3.84	0.15	0.42	0.25	7	16	44	150	-
	1.53	4.00	0.15	0.42	0.26	7	18	46	150	-
Final coarse fraction retained by sieve # 12	3.72	6.00	0.18	0.40	0.27	8	22	50	190	277
	3.64	5.91	0.19	0.40	0.28	9	23	50	180	297
Final coarse fraction retained by sieve # 20	4.08	6.30	0.20	0.40	0.29	8	26	48	150	316
	3.99	6.00	0.20	0.42	0.30	10	26	48	140	273
Final coarse fraction retained by sieve # 35	2.53	3.56	0.16	0.36	0.23	18	16	72	180	128
	2.55	3.32	0.16	0.34	0.22	12	16	87	190	124
Final fine fraction of wet screening	2.55	2.50	0.19	0.38	0.26	12	18	67	150	50
	2.58	2.95	0.20	0.40	0.23	14	17	65	220	48
Low density fraction of air classification	4.53	7.07	0.22	0.50	0.27	7	25	52	140	160
	4.98	6.98	0.22	0.50	0.28	8	28	54	140	181

Table 39

Mineral Composition of the Products of
Wet Screening pH-10, Run Two

Products	P %	Ca %	Mg %	K %	Na %	Cu ppm	Mn ppm	Zn ppm	Fe ppm	F ppm
Raw Product- wet basis	0.67	1.11	0.04	0.24	0.12	2.5	4.2	11	42	25
Solids of raw product	2.15	3.69	0.12	0.80	0.38	8	14	37	140	78
	2.14	3.43	0.12	0.74	0.38	8	13	34	130	81
Solids of first extraction	2.42	3.43	0.13	0.42	0.24	6	12	34	150	-
	2.26	3.43	0.13	0.42	0.24	4	13	34	170	-
Solids of second extraction	2.68	5.02	0.16	0.36	0.22	5	19	38	180	-
	2.81	5.30	0.11	0.36	0.22	7	18	38	180	-
Final coarse fraction retained by sieve # 12	3.48	5.38	0.19	0.37	0.25	6	21	47	200	242
	3.37	5.57	0.18	0.38	0.24	10	20	62	180	226
Final coarse fraction retained by sieve # 20	3.34	5.38	0.19	0.34	0.24	6	21	40	180	225
	3.34	5.42	0.19	0.34	0.25	6	22	40	180	196
Final coarse fraction retained by sieve # 35	2.83	4.27	0.19	0.34	0.24	45	20	56	300	131
	2.77	4.23	0.19	0.36	0.23	40	18	52	330	128
Final fine fraction of wet screening	1.78	2.39	0.20	0.34	0.19	24	15	72	470	40
	1.79	2.45	0.19	0.36	0.19	19	15	72	480	41
Low density fraction of air classification	5.00	7.22	0.24	0.48	0.36	25	26	73	190	196
	4.99	7.30	0.25	0.50	0.36	18	25	60	190	186

Table 40

Mineral Composition of the Products of
Wet Screening pH-10, Run Three

Products	P %	Ca %	Mg %	K %	Na %	Cu ppm	Mn ppm	Zn ppm	Fe ppm	F ppm
Raw Product- wet basis	0.70	1.02	0.03	0.25	0.11	1.9	4.1	10	49	28
Solids of raw product	2.40	3.63	0.14	0.89	0.38	6	14	34	160	96
	2.34	3.32	0.13	0.81	0.40	7	14	31	170	95
Solids of first extraction	2.58	4.80	0.15	0.54	0.26	6	19	38	180	-
	2.80	4.50	0.16	0.56	0.27	4	20	40	170	-
Solids of second extraction	2.03	5.27	0.14	0.38	0.24	9	17	39	170	-
	2.11	5.26	0.13	0.39	0.23	8	19	41	190	-
Final coarse fraction retained by sieve # 12	3.46	5.38	0.19	0.46	0.27	9	24	49	200	234
	3.50	5.35	0.20	0.46	0.28	7	24	52	210	233
Final coarse fraction retained by sieve # 20	3.79	6.00	0.18	0.46	0.28	6	27	49	180	215
	3.83	6.10	0.20	0.44	0.30	7	28	49	210	241
Final coarse fraction retained by sieve # 35	3.26	4.52	0.20	0.42	0.27	28	24	52	200	164
	3.32	4.70	0.20	0.40	0.27	32	24	56	220	176
Final fine fraction of wet screening	1.95	2.67	0.21	0.38	0.20	29	24	62	500	42
	1.97	2.53	0.19	0.34	0.20	28	24	67	490	42
Low density fraction of air classification	4.56	7.28	0.30	0.63	0.49	18	19	57	160	228
	4.66	7.45	0.30	0.62	0.50	19	18	57	170	232

Table 41

Model Analysis of Variance Table
for Sets 1, 2, 3 and 4

Source of variation	d.f. Set 1	d.f. Set 2	d.f. Set 3	d.f. Set 4
Treatments	3	2	3	2
Products	6	7	3	4
Prod x treat	18	14	9	8
Run x prod x treat[1]	58	48	32	30
Dup x run x prod x treat	84	72	48	45
Corrected total	167	143	95	89

1 Residual.

VITA

Javier Loustaunau was born on February 1, 1944 in San Luis Potosi, Mexico, the son of Jose C. Loustaunau and Consuelo T. Loustaunau. He attended primary and secondary school in the Instituto Potosino. In January, 1961 he entered the Instituto Tecnologico de Monterrey where he received his Preparatory degree 1963, and in January, 1969 a B.S. in Biochemical Engineering specializing in Food Technology.

In February, 1969, he entered the Graduate School of Louisiana State University in the Department of Food Science from which he received a Master of Science degree in January, 1971. He married Jane M. Hotard in September of 1969. He is presently a candidate at Louisiana State University for the degree of Doctor of Philosophy in Food Science.

EXAMINATION AND THESIS REPORT

Candidate: Javier Loustaunau

Major Field: Food Science

Title of Thesis: "Characteristics of a Low Fluoride Fish Protein Concentrate from Whole Croaker (Micropogon undulatus)"

Approved:

Fred H. Hoobens
Major Professor and Chairman

James E. Trayngham
Dean of the Graduate School

EXAMINING COMMITTEE:

Arthur F. Novak

William H. Jones

James E. Rutledge

William J. Mitchell

Samuel Meyer

Date of Examination:

July 20, 1973